Vol. V 1935

THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE

Issued under the Authority
of
The Imperial Council of Agricultural Research

Annual subscription
Rs. 15 or 24s.

Price per part
Rs. 3 or 5s. 3d.

PUBLISHED BY MANAGER OF PUBLICATIONS, DELHI
PRINTED BY THE MANAGER, GOVERNMENT OF INDIA PRESS, NEW DELHI
1937.

Price Annas 4 or 5d.

List of Agents in India from whom

Government of India Publications are available.

(*a*) Provincial Government Book Depots.

Madras:—Superintendent, Government Press, Mount Road, Madras.

Bombay:—Superintendent, Government Printing and Stationery, Queen's Road, Bombay.

Sind:—Manager, Sind Government Book Depot and Record Office, Karachi (Sadar).

United Provinces:—Superintendent, Government Press, Allahabad.

Punjab:—Superintendent, Government Printing, Punjab, Lahore.

Burma:—Superintendent, Government Printing, Burma, Rangoon.

Central Provinces:—Superintendent, Government Printing, Central Provinces, Nagpur.

Assam:—Superintendent, Assam Secretariat Press, Shillong.

Bihar:—Superintendent, Government Printing, P. O. Gulzarbagh, Patna

North-West Frontier Province:—Manager, Government Printing and Stationery, Peshawar.

Orissa:—Press Officer, Secretariat, Cuttack.

(*b*) Private Book-sellers.

Advani Brothers, P. O. Box 100, Cawnpore.
Aero Stores, Karachi.*
Banthiya & Co., Ltd., Station Road, Ajmer.
Bengal Flying Club, Dum Dum Cantt.*
Bhawnani & Sons, New Delhi.
Book Company, Calcutta.
Booklover's Resort, Taikad, Trivandrum, South India.
Burma Book Club, Ltd., Rangoon.
Butterworth & Co. (India), Ltd., Calcutta.
Careers, Mohini Road, Lahore.
Chatterjee & Co., 3, Bacharam Chatterjee Lane, Calcutta.
Chukervertty, Chatterjee & Co., Ltd., 13, College Square, Calcutta.
Das Gupta & Co., 54/3, College Street, Calcutta.
Delhi and U. P. Flying Club, Ltd., Delhi.*
English Book Depot, Ferozepore.
English Bookstall, Karachi.
Faqir Chand Marwah, Peshawar Cantonment.
Fono Book Agency, Simla.
Higginbothams, Madras.
Hindu Library, 137/F, Balaram De Street, Calcutta.
Hyderabad Book Depot, Chaderghat, Hyderabad (Deccan).
Imperial Book Depot and Press, near Jama Masjid (Machhliwalan), Delhi.
Indian Army Book Depot, Dayalbagh, Agra.
Indian Army Book Depot, Daryaganj, Delhi.
Indian Book Shop, Benares City.
Indian School Supply Depot, Central Avenue South, P. O. Dharamtala, Calcutta.
Insurance Publicity Co., Ltd., Lahore.
International Book Service, Poona 4.
Jacques & Co., Kamptee Road, Nagpur, Messrs. Neston.
Jaina & Bros., Mori Gate, Delhi, and Connaught Place, New Delhi, Messrs. J. M.
Kamala Book Depot, 15, College Square, Calcutta.
Karnataka Publishing House, Bangalore City.
Keale & Co., Karachi.
Kitabistan, 17-A, City Road, Allahabad.
Krishnaswami & Co., Teppakulam P. O., Trichinopoly Fort, Messrs. S.
Lahiri & Co., Calcutta, Messrs. S. K.
Local Self-Govt. Institute, Bombay.
London Book Co. (India), Arbab Road, Peshawar, Murree, Nowshera, Rawalpindi.
Messrs. U. P. Malhotra & Co., Post Box No. 94, Lahore.
Minarva Book Shop, Anarkali Street, Lahore.
Modern Book Depot, Bazar Road, Sialkot Cantonment.
Modern Book Depot, Napier Road, Jullundur Cantt.
Mohanlal Dossabhai Shah, Rajkot.
Nandkishore & Bros., Chowk, Benares City.
New Book Co., "Kitab Mahal," 192, Hornby Road, Bombay.
Newman & Co., Ltd., Calcutta, Messrs. W.
Oxford Book and Stationery Company, Delhi, Lahore, Simla, Meerut and Calcutta.
Parikh & Co., Baroda, Messrs. B.
Pioneer Book Suppiy Co., 20, Shib Narayan Das Lane Calcutta, and 219, Cloth Market, Delhi.
Popular Book Depot, Grant Road, Bombay.
Punjab Religious Book Society, Lahore.
Raghunath Prasad & Sons, Patna City.
Rama Krishna & Sons, Booksellers, Anarkali, Lahore.
Ram Krishna Bros., Opposite Bisnrambag, Poona City.
Ram Narain Lal, Katra, Allahabad.
Ramesh Book Depot and Stationery Mart, Kashmere Gate, Delhi.
Ray & Sons, 43, K. & L. Edwardes Road, Rawalpindi Murree and Peshawar, Messrs. J.
Roy Chowdhury & Co., 72, Harrison Road, Calcutta, Messrs. N. M.
Sarcar & Sons, 15, College Square, Calcutta, Messrs. M. C.
Sarkar & Co., Ltd., 18, Shama Charan De Street, and 8/2, Hastings Street, Calcutta, Messrs. P. C.
Standard Bookstall, Karachi.
Standard Bookstall, Quetta.
Standard Book Depot, Lahore, Dalhousie and Delhi.
Standard Law Book Society, 69, Harrison Road, Calcutta.
Taraporevala Sons & Co., Bombay, Messrs. D. B.
Thacker & Co., Ltd., Bombay.
Thacker, Spink & Co., Ltd., Calcutta and Simla.
Tripathi & Co., Booksellers, Princes Street, Kalbadev Road, Bombay, Messrs. N. M.
University Book Agency, Kacheri Road, Lahore.
Upper India Publishing House, Ltd., Literature Palace Ammuddaula Park, Lucknow.
Varadachary & Co., Madras, Messrs. P.
Venkatasubban, A., Law Bookseller, Vellore.
Wheeler & Co., Allahabad, Calcutta and Bombay, Messrs. A. H.
Young Man & Co., Ajmer and Egerton Road, Delhi.

* Agent for publications on Aviation only.

CONTENTS OF VOLUME V

PART I (FEBRUARY, 1935)

ORIGINAL ARTICLES

NOTES

PART II (APRIL, 1935)

ORIGINAL ARTICLES

NOTE

PART III (JUNE, 1935)

ORIGINAL ARTICLES

PART IV (AUGUST, 1935)

ORIGINAL ARTICLES

NOTES

PART V (OCTOBER, 1935)

ORIGINAL ARTICLES

INDEX TO VOLUME V

A

B

G

H

R

S

ERRATA

INDIAN JOURNAL OF AGRICULTURAL SCIENCE VOL. V, PART I, FEBRUARY 1935

Page 101, lines 2-5 should read as follows :—

E. A. OSADCHUK. (*Juzno-bereznoe Otdelenie V. I. R'a Gosudarstennyi Nikitskii Botaniceskii Sad*, (*Nikita Botanic Garden*) 1934 : *Bull. No.* 12, *pp.* 40. (*Full summary of the paper, issued by the Imperial Bureau of Plant Genetics, School of Agriculture, Cambridge, England*).

Page 103, item 10 of table 3, *for* " Persican " *read* " Persican ".

Page 103, item 13 of table 3, *for* " Kizildza Mursal " *read* " Kizildza Mursal "

GIPD—267ICofAR—S3—14-5-37—800.

NOTICE

The Indian Journal of Agricultural Science

A Bi-monthly Scientific Journal of Agriculture and the Allied Sciences, mainly devoted to the publication of the results of original research and field experiments

---

Contributions should be sent to the Secretary, Imperial Council of Agricultural Research (Publication Section), New Delhi, India. Contributors are invited to sign their contributions. All communications must be authenticated by the name and address of the contributor, whether intended for publication or not.

The attention of authors is invited to the 'Instructions to authors of publications of the Imperial Council of Agricultural Research', printed in the February (1935) Number of this Journal. Spare copies of the Instructions can be had on application to the Secretary, Imperial Council of Agricultural Research (Publication Section), New Delhi.

Contributors of original articles receive 25 copies of their contributions free and can purchase additional copies if notice be given when their proofs are returned. The cost of additional reprints will vary according to the number required; the following are approximate rates for printing 50 copies over the 25 copies supplied free:— As. 8-6 per type page, As. 15-3 per black and white plate and Rs. 2-8-0 per three-coloured plate.

Copies of the journal can be had from the Manager of Publications, Civil Lines, Delhi, and Agents for all Government publications. **Annual subscription Rs. 15 or 24s. inclusive of Indian postage, payable in advance. Price per part Rs. 3 or 5*s.* 3*d.* inclusive of Indian postage** All communications regarding subscription and advertisements should be addressed to the Manager of Publications, Civil Lines, Delhi.

ORIGINAL ARTICLES

*AGRICULTURAL METEOROLOGY: STUDIES IN MICRO-CLIMATOLOGY, PART II

BY

L. A. RAMDAS, M.A., PH.D.,

Agricultural Meteorologist, Poona,

R. J. KALAMKAR, B Sc., B.AG., PH.D.,

AND

K. M. GADRE, B.AG.

(Received for publication on 24th September 1934)

INTRODUCTION

In Part I of this series [Ramdas, Kalamkar and Gadre, 1934] the scope of "micro-climatology" was summarised. This was followed by a brief discussion of different types of instruments for the measurement of air temperature and humidity. Mention was also made of micro-climatic observations taken in the 'open' and inside *'Kharif' jowar* and sugarcane during the first half of December 1932 at the Agricultural Meteorological Observatory, Poona.

These observations were taken inside a few crops and in the 'open' daily at the epochs of maximum and minimum temperature. Results of observations during part of the *Rabi* season of the year 1932-33 are discussed in the present paper and an attempt is made to investigate the relationship, if any, between the observations taken inside the crops with those in the 'open'.

The *Rabi* season, October to March or April, is, as mentioned earlier in Part I, a period of clear weather with light winds constituting a climate predominantly of the insolation or radiation type. This season therefore deserves first consideration in any programme of micro-climatic investigations.

We shall discuss here the dry and wet bulb temperatures as well as the pressure of water vapour and percentage humidity calculated from them, with a view to study the modifying influence of different crops on the climate of the open, within the first few feet above ground up to which crops usually grow. While the conditions during individual seasons may vary from year to year it

*The investigations discussed in this series were made in the Agricultural Meteorology Branch (India Meteorological Department), financed by the Imperial Council of Agricultural Research.

may be expected that certain main regularities may become evident during an examination of the data collected even during an individual season.

The role of the humidity factor in micro-climates will be discussed in detail in the next part of this series.

II. Micro-climate of crops and of the open

Micro-climate in the 'open' and inside the following crops are discussed:—

(*a*) *Rabi jowar.*

(*b*) Sugarcane.

(*c*) Wheat.

Details regarding the dates of sowing, variety, seed rate, cultural operations, dates of cropping etc. of the above crops are given in Table I.

TABLE I

Particulars	*Kharif jowar*	Sugarcane	*Rabi jowar*	Wheat
Date of sowing . .	19th July 1932.	1st Feby. 1932.	7th Oct. 1932.	15th Oct. 1932.
Seed rate per acre .	8 lbs.	....	7 lbs. (18″ apart).	40 lbs. (1′ apart).
Variety . . .	Gidgap.	Khadya.	Maldandi.	Parner.
Germination . .	25th July 1932.	17th Feby. 1932.	13th Oct. 1932.	20th Oct. 1932.
Plant density per square yard.	12	11 with tillers.	12	126 shoots.
Dates of irrigation (during period under study).	Unirrigated.	Irrigated on 8, 12 December.	Unirrigated.	Unirrigated.
Date of flowering . .	12th Nov. 1932.	30th Nov. 1932.	22nd Dec. 1932.	24th Dec. 1932.
Earthing up . .	....	10th July 1932.	....	....
Date of harvest . .	15th Dec. 1932.	3rd Jan. 1933.	17th Feb.1933.	24th Feb. 1933.
Average height . .	5-8-32—1 to 5″. 20-8-32—6 to 7″. 7-9-32—3′ to 4′. 21-9-32—4′ to 6′. 5-10-32—6′ to 10′. 21-10-32—8′ to 10′.	23-3-32—5″. 8-4-32—6″. 21-4-32—6 5″. 2-5-32—8″. 24-5-32—10″. 4-6-32—15″. 1-7-32—19″. 10-7-32—3′ 7″. 2-12-32—8′ 5″.	1-11-32—6 to 8″. 15-11-32—10 to 16″. 29-11-32—3½ to 4′. 12-12-32—4½ to 5½′. 27-12-32—5 to 6′. 25-1-33—5 to 6′ 9″	27-10-32—6 to 7″. 12-11-32—9 to 10″. 3-12-32—12 to 14″. 15-12-32—15 to 18″ tillers 4-6. 24-12-32—24″. 12-1-33—3′ 6″.

For inter-comparison the temperature and humidity data have been studied at Poona during the following periods:—

(1) Open, sugarcane, *Kharif-jowar*, 4th—15th December 1932.
(2) Open, sugarcane, *Rabi-jowar*, 16th—31st December 1932.
(3) Open, *Rabi jowar*, wheat, 8th—31st January 1933.
(4) Open, *Rabi jowar*, wheat, 1st—21st February 1933.

Some of the periods 1 to 4 could of course be combined. This has not been done in view of the fact that the *Rabi* crops were in a state of growth and it was considered desirable to keep the number of days in each group as small as possible.

The means of the observations of dry bulb, wet bulb, vapour pressure and humidity percentage for the different periods (except the first one for which data have already been given in Part I) are given in Tables II-IV for the minimum and maximum temperature epochs.

The contents of Tables II-IV confirm the conclusions of Part I of this series. The influence of the nature of the crop and its height on micro-climate will be seen from the following pages.

(*a*) *Maximum temperature epoch*

(*1*) *Dry bulb temperature.*—It may be observed from Tables II-IV that at the maximum epoch the dry bulb temperature in the 'open' is very high near the ground and falls rapidly with height. Dry bulb temperatures in *jowar* (an unirrigated crop) near the ground are lower than in the open but at higher levels they approach those of the open and in later stages of growth even exceed slightly the dry bulb temperatures at correspondnig heights in the 'open' (as seen in Tables III and IV). In the case of sugarcane (an irrigated crop) the dry bulb temperatures near the soil are much lower than in *jowar*, and they have a tendency to increase with height; but even at 6 ft. the temperature is about a degree lower than that of the 'open'. The largest difference between the 'open' and sugarcane is about 14°C. near the soil, 3·5°C. at 2 ft. and 2°C. at 4 ft. In the case of the wheat crop (Tables III and IV) the dry bulb temperatures are comparatively higher than that of the 'open' at similar heights; this may be due to the fact that there is ineffective shading of the ground which receives a large amount of insolation and that at the same time air movements or "convective processes" are suppressed by the vegetation.

TABLE II

Mean dry bulb and wet bulb temperatures, vapour pressure and humidity percentage during the period 16th to 31st December 1932

"Dry Continental Type."

Height	Time	Dry bulb °C.			Wet bulb °C.			Vapour pressure in mm. of mercury			Humidity percentage		
		Open	*Rabi jowar*	Sugar-cane	Open	*Rabi jowar*	Sugar-cane	Open	*Rabi jowar*	Sugar-cane	Open	*Rabi jowar*	Sugar-cane
0·3″	Morning (Minimum Epoch.)	7·7	8·3	9·2	5·2	6·6	8·4	5·4	6·5	7·9	68	78	90
1″		7·3	8·1	8·9	5·0	6·5	8·2	5·4	6·5	7·8	70	81	91
3″		7·1	7·8	8·4	4·9	6·3	7·7	5·5	6·5	7·6	71	81	91
6″		6·9	7·6	8·1	5·0	6·2	7·4	5·6	6·5	7·4	74	81	92
1 ft.		7·1	7·4	7·9	5·3	6·2	7·3	5·9	6·5	7·4	77	84	92
2 ft.		7·4	7·2	7·9	5·8	6·1	7·3	6·2	6·5	7·4	79	85	91
3 ft.		7·8	7·3	8·1	6·3	6·2	7·4	6·5	6·7	7·4	81	86	91
4 ft.		8·3	7·5	8·2	6·9	6·4	7·4	6·8	6·7	7·4	82	86	90
6 ft.		8·7	8·0	8·4	7·2	6·8	7·6	6·9	6·9	7·4	82	84	89
0·3″	Afternoon (Maximum Epoch.)	35·8	30·3	22·4	18·7	18·4	17·2	7·6	9·9	12·2	17	31	60
1″		34·0	30·3	22·8	17·8	17·9	17·1	7·3	9·2	11·8	18	28	56
3″		32·9	29·9	23·3	17·4	17·4	16·8	7·2	8·7	11·3	19	27	53
6″		31·6	29·7	24·0	16·8	17·3	16·6	7·0	8·5	10·5	20	27	47
1 ft.		30·8	29·5	24·8	16·5	16·9	16·2	7·0	8·2	9·5	21	27	41
2 ft.		29·7	29·1	26·2	16·1	16·7	15·7	7·0	8·0	8·2	22	27	32
3 f		29·6	28·9	27·0	16·0	16·5	15·6	6·9	7·9	7·6	22	26	29
4 ft.		29·	28·6	27·2	15·7	16·2	15·5	6·8	7·7	7·4	23	26	27
6 ft.		28·8	28·4	27·7	15·6	16·1	15·5	6·7	7·5	7·1	23	26	26

TABLE III

Mean dry bulb and wet bulb temperatures, vapour pressure and humidity percentage during the period 8th to 31st January 1933

"Dry Continental Type."

Height	Time	Dry bulb °C.			Wet bulb °C.			Vapour pressure in mm. of mercury			Humidity percentage		
		Open	*Rabi jowar*	Wheat	Open	*Rabi jowar*	Wheat	Open	*Rabi jowar*	Wheat	Open	*Rabi jowar*	Wheat
0·3″	Morning (Minimum Epoch.)	9·4	9·7	8·7	6·3	8·0	7·3	5·7	7·3	7·1	63	79	82
1″		9·2	9·6	8·5	6·3	8·0	7·2	5·8	7·4	7·1	65	81	84
3″		8·9	9·4	8·1	6·1	7·9	7·1	5·7	7·3	7·2	65	81	87
6″		8·7	9·3	7·9	6·0	7·8	7·1	5·9	7·3	7·4	68	82	90
1 ft.		8·7	9·1	7·8	6·4	7·7	7·1	6·3	7·4	7·4	72	82	91
2 ft.		9·1	9·1	8·2	6·9	7·7	7·5	6·5	7·4	7·6	73	84	91
3 ft.		9·6	9·1	9·2	7·4	7·8	7·9	6·8	7·4	7·5	74	84	85
4 ft.		9·9	9·2	9·7	7·8	7·8	8·1	7·0	7·4	7·5	75	83	81
6 ft.		10·4	9·9	10·4	8·3	8·3	8·6	7·3	7·5	7·6	76	81	79
0·3″	Afternoon (Maximum Epoch.)	36·2	33·2	36·3	19·5	20·1	20·5	8·7	10·6	10·3	19	27	23
1″		34·2	32·4	35·5	18·3	18·8	20·0	7·9	9·6	9·9	19	26	23
3″		32·9	31·7	34·5	17·9	18·4	19·5	7·9	9·2	9·6	20	26	23
6″		31·8	31·2	33·6	17·3	18·0	18·8	7·7	8·9	8·9	22	26	23
1 ft.		30·9	31·0	32·9	17·0	17·9	18·4	7·7	8·9	8·7	22	26	23
2 ft.		30·1	30·5	31·8	16·7	17·5	17·9	7·7	8·6	8·5	23	26	24
3 ft.		29·6	30·0	30·7	16·5	17·1	17·3	7·6	8·3	8·1	24	26	25
4 ft.		29·2	29·5	30·1	16·4	16·8	16·8	7·6	8·1	7·8	25	26	24
6 ft.		28·7	29·2	29·4	16·1	16·5	16·3	7·5	7·8	7·5	25	25	24

TABLE IV.

Mean dry bulb and wet bulb temperatures, vapour pressure and humidity percentage during the period 1st to 21st February 1933

"Dry Continental Type."

Height	Time	Dry bulb °C.			Wet bulb °C.			Vapour pressure in mm. of mercury			Humidity percentage		
		Open	*Rabi jowar*	Wheat	Open	*Rabi jowar*	Wheat	Open	*Rabi jowar*	Wheat	Open	*Rabi jowar*	Wheat
0·3″	Morning (Minimum Epoch.)	11·5	11·8	10·4	7·9	9·2	8·4	6·3	7·2	7·3	61	68	77
1″		11·3	11·8	10·2	7·6	9·2	8·2	6·0	7·5	7·3	59	71	77
3″		10·9	11·5	9·9	7·4	9·0	8·2	6·1	7·4	7·3	61	72	79
6″		10·6	11·3	9·8	7·3	8·9	8·2	6·1	7·4	7·5	64	73	81
1 ft.		10·7	11·1	9·8	7·7	8·8	8·2	6·4	7·5	7·4	66	75	81
2 ft.		11·1	11·0	10·2	8·3	8·8	8·5	6·8	7·5	7·5	68	75	79
3 ft.		11·4	11·3	11·1	8·6	9·0	9·0	7·1	7·5	7·6	69	75	76
4 ft.		11·7	11·5	11·7	8·8	9·1	9·3	7·2	7·6	7·6	69	74	73
6 ft.		12·3	12·1	12·3	9·5	9·6	9·7	7·5	7·8	7·8	70	73	72
0·3″	Afternoon (Maximum Epoch.)	39·5	37·7	42·0	19·2	19·5	20·9	6·5	7·9	8·1	12	16	14
1″		37·4	36·4	41·0	18·3	18·7	20·3	6·2	7·0	7·6	13	15	13
3″		36·0	35·5	39·6	17·8	18·0	19·6	6·2	6·7	7·2	13	16	13
6″		34·6	34·7	38·5	17·1	17·6	19·1	5·9	6·5	6·9	15	16	14
1 ft.		33·6	34·0	37·1	16·8	17·3	18·4	5·9	6·4	6·0	15	16	13
2 ft.		33·0	33·1	35·5	16·5	16·7	17·7	6·0	6·1	6·3	15	16	14
3 ft.		32·2	32·6	33·9	16·2	16·7	17·1	5·9	6·3	6·2	16	17	16
4 ft.		32·0	32·1	32·9	16·1	16·3	16·5	5·8	6·0	5·8	16	17	16
6 ft.		31·5	32·0	32·2	15·8	16·1	16·2	5·6	5·8	5·8	16	16	16

(2) *Wet bulb temperature.*—The wet bulb temperatures at the maximum epoch show a decrease with height though less conspicuously than the dry bulb temperatures. The wet bulb temperatures in the 'open' are in general slightly lower than inside *jowar* or wheat (un-irrigated), but in the case of sugarcane (Table II) the values are lower than in the open. Inside wheat (Tables III and IV) the wet bulb temperatures are slightly warmer than inside *jowar*.

(3) *Vapour pressure.*—Vapour pressure inside the crops is higher at all heights compared to the 'open', the highest values being recorded inside sugarcane. There is a tendency for the vapour pressure to decrease with height both in the open and inside the crops.

(4) *Percentage humidity.*—The percentage humidity is minimum near the ground and increases with height in the 'open', the degree of saturation being least in the very warm air layers near the ground.

(*b*) *Minimum temperature epoch*

(1) *Dry bulb temperature.*—As observed in Part I although the variation of micro-climates is less at this epoch, some very interesting features are present. The dry bulb temperatures decrease at first with height both in the 'open' and inside the crops and then begin to increase, the level of inversion being about 6 in. in the 'open', about 1 ft. inside wheat and about 2 ft. inside *jowar* and sugarcane. Temperatures inside tall crops like *jowar* and sugarcane are higher than in the 'open' but tend to the open air value at higher levels. It is remarkable that in the case of the sugarcane field (Table II) which is coolest during the maximum temperature epoch, the minimum temperatures are higher than in the open or inside *jowar*. Inside wheat (a short crop) the minimum temperatures are lower than either in the 'open' or in *jowar*. The explanation of these variations as well as of the coolest air being at some height above ground will be discussed as soon as certain investigations on radiation are completed. They are of special interest in connection with the incidence of frost during winter. Replies to a questionnaire on the frosts which occurred during the last winter lend some support to the generality of the above phenomenon, the problem of frost hazard in India as well as the possible remedial measures to minimise damage are being examined in the light of the various detailed reports received this year. An analysis of the daily minimum temperatures during winter at representative stations in India is also in progress in this connection. Further experimental work at different stations is essential for studying how the frequency of frost varies with height above ground and with locality.

(2) *Wet bulb temperature.*—The wet bulb temperatures at the minimum epoch do not differ very much from the corresponding dry bulb temperatures. The inversions of wet bulb temperature occur more or less at the same height above ground as the latter. The wet bulb temperatures in the 'open' are lower than those inside crops; those in sugarcane are higher than in other crops.

(3) *Vapour pressure.*—Vapour pressure in the 'open' is throughout less than that inside the crops. There is a tendency for the vapour pressure to increase with height in the 'open' and inside *jowar* and wheat. Inside sugarcane, however, it decreases slightly with height in the first foot and is more or less steady at higher levels.

The physical significance of the increase in the vapour pressure with height during night and the reversal of the phenomenon during day has been discussed by Ramdas [1934] and Ramdas and Katti [1934, 1 & 2] in separate papers where the decrease of moisture near the ground at night has been shown to be due to absorption by the soil surface and the reverse effect during day to evaporation from the soil.

(4) *Percentage humidity.*—From Tables II, III and IV it will be noticed that the percentage humidity or the degree of saturation of air with water vapour is lower in the open than inside the crops. In the 'open' as well as inside *jowar* there is a regular increase with height above the ground. In sugarcane (irrigated) the saturation is maximum and more or less uniform at all levels up to 4 feet above which there is a tendency to fall. Inside wheat the percentage is higher than in *jowar* but above 2 feet (the approximate height of the crop) it decreases to the open air values.

III. Correlations between "open" and "crop" temperatures and humidities.

Having seen that the micro-climate of the crops is different from that of the 'open', it is next attempted to investigate whether the micro-climates inside the crops are correlated with conditions in the open.

The values of correlation co-efficients of some of the factors, dry bulb, wet bulb, and vapour pressure for various heights inside the crops with those at corresponding heights in the open are given in Tables V, VI, VII and VIII, both for the minimum and maximum epochs.

Table V

Correlation between temperatures (4th to 31st December 1932)

[For 5 per cent level of significance $r = 0{\cdot}38$]

In the open	With those taken in sugarcane	
	Morning	Afternoon
Surface	·93	·59
1″	·92	·61
3″	·92	·47
6″	·92	·47
1 ft.	·91	·45
2 ft.	·93	·23
3 ft.	·93	·59
4 ft.	·90	·69
6 ft.	·85	·63

TABLE VI

Correlation r between dry and wet bulb temperatures and pressure of water vapour

[For 5 per cent level of significance $r=0\cdot58$]

	In the open	With those taken in (4th to 15th December 1932)					
		Dry bulb		Wet bulb		Vapour pressure	
		Jowar	Sugarcane	*Jowar*	Sugarcane	*Jowar*	Sugarcane
Morning.	Surface .	·91	·85	·82	·89	·72	·83
	6″ .	·92	·91	·92	·93	·90	·92
	1 ft. .	·94	·96	·94	·95	.93	·93
	2 ft. .	·94	·98	·90	·97	·89	·95
	4 ft. .	·94	·91	·94	·93	·92	·91
	6 ft. .	·91	·87	·91	·93	·89	·90
Afternoon.	Surface .	·56	·64	·91	·17	·91	·16
	6″ .	·73	·53	·65	·19	·51	·12
	1 ft. .	·59	·50	·60	·56	·78	·33
	2 ft. .	·67	·48	·73	·60	·75	·68
	4 ft. .	·84	·85	·69	·74	·70	·85
	6 ft. .	·82	·78	·86	·81	·85	·91

TABLE VII.

Correlation r between dry and wet bulb temperatures and pressure of water vapour

[For 5 per cent level of significance $r=0\cdot40$]

	In the open	With those taken in (8th to 31st January 1933)					
		Dry bulb		Wet bulb		Vapour pressure	
		Jowar	Wheat	*Jowar*	Wheat	*Jowar*	Wheat
Morning.	Surface .	·98	.98	·97	·97	·93	·94
	6″ .	·98	.97	·96	·96	·91	·92
	1 ft. .	·96	.97	·95	·95	·91	·89
	2 ft. .	·97	.97	·96	·95	·92	·89
	4 ft. .	·92	.89	·93	·95	·93	·93
	6 ft. .	·97	.95	·96	·95	·93	·95
Afternoon.	Surface .	·70	·31	·55	·84	·70	·82
	6″ .	·77	·61	·98	·92	·92	·94
	1 ft. .	·70	·59	·75	·80	·97	·94
	2 ft. .	·72	·67	·91	·93	·99	·94
	4 ft. .	·80	·78	·93	·94	·96	·94
	6 ft. .	·84	·83	·95	·97	·97	·97

TABLE VIII

Correlation r between dry bulb and wet bulb temperatures

[For 5 per cent level of significance $r=0{\cdot}43$]

	In the open	With those taken in (1st to 21st February 1933)			
		Dry bulb		Wet bulb	
		Jowar	Wheat	*Jowar*	Wheat
Morning.	Surface	·86	·88	·91	·89
	6″	·91	·91	·94	·91
	1 ft.	·90	·93	·90	·88
	2 ft.	·92	·89	·92	·89
	4 ft.	·90	·91	·95	·95
	6 ft.	·92	·87	·73	·91
Afternoon.	Surface	·36	·05	·79	·58
	6″	·52	·27	·74	·64
	1 ft.	·37	·37	·77	·73
	2 ft.	·46	·54	·64	·65
	4 ft.	·66	·71	·87	·59
	6 ft.	·83	·60	·92	·88

It may be observed that the morning correlations of all the meteorological elements are uniformly high, whereas those for the maximum epoch are smaller especially at lower levels. In the afternoon the correlation improves at higher levels. These results suggest that temperature and humidity inside crops are not related in a simple manner to those in the 'open' and that it may be necessary to measure them directly if one desires to study the actual climatic conditions inside the crops. Further studies on these lines and for different crops are being continued.

Our best thanks are due to the Principal of the Agricultural College, Poona, for giving necessary facilities for this work in the College farm.

REFERENCES

Ramdas, L. A. (1934). *Current Sci.* **2**, 4454-7.

Ramdas, L. A.; Kalamkar R. J. and Gadre, K. M. (1934). *Ind. J. Agric. Sci.* **4**, 451-67.

Ramdas, L. A. and Katti, M. S. (1934, 1). *Current Sci.* **3**, 24.

——————————————(1934, 2). *Ind. J. Agric. Sci.* **4**, 923-37.

A BIOCHEMICAL STUDY OF THE STARCHES FROM OLD AND NEW GRAIN OF DIFFERENT VARIETIES OF RICE

BY

D. L. SAHASRABUDDHE, M.Ag., M.Sc.,

Agricultural Chemist to Government, Bombay, Poona,

AND

M. M. KIBE, M.Ag., B.Sc.

(Received for publication on 3rd October 1934)

I. Introduction

It is supposed that rice of some varieties is fit for being used as boiled rice while that of other varieties is more suited for bread-making than for boiling. There is also a supposition that old rice is more easily digested than new rice. Since rice grain is chiefly made up of starch, and proteids and oil are present only to a very small extent, the differences on account of the varieties and ages must naturally be due mainly to differences in the starchy material. It may be that different varieties of rice contain starches with different reacting powers. It is also quite possible that when rice is stored the starch in the grain undergoes slow change and disintegration, making the whole grain more and more easily digestible with the increase in period of storage.

In order to ascertain whether there are any varietal differences in the appearance and reaction of rice starches and whether during storage the rice starch undergoes any disintegration a regular experimental programme was decided upon. The varieties of rice included in the study were (1) Ambemohor, (2) Kolamba, (3) Patni, (4) Halwar and (5) Mahadi. In studying the differences due to age, rice harvested at the end of 1928 and that at the end of 1930 was taken. The work on age difference took some months but all along there was a difference of two years between the old and the new. In addition, there were also some samples or rice one-year, two-year, and four-year old. Description of the varieties of rice used in these experiments are given in a tabular form as under:—

TABLE I

Description of rice varieties

	Ambemohor	Kolamba	Patni	Halwar	Mahadi
Size— Length . .	6·6 mm.	7·4 to 8·3 mm.	8·2 mm.	8·5 mm.	9·7 mm.
Breadth .	2·7 mm.	2·4 to 2·5 mm.	3·0 mm.	2·8 mm.	3·8 mm.
Colour of husk .	Brownish to reddish yellow.	Golden to pale yellow.	Pale yellow to whitish.	Pale yellow	Yellow husk and reddish grain.
Grade . .	Medium to fine.	Medium to fine.	Medium coarse.	Medium coarse.	Coarse.
Use . . .	Boiled rice.	Boiled rice.	Boiled rice and bread-making.	Boiled rice and bread-making.	Bread making and cattle feed.
Habitat . .	Maval tract of the Deccan.	North Konkan.	North Konkan.	North Konkan.	North Konkan.

The investigation was carried on along the following lines:—

1. Some physical properties of rice starch such as size of starch grain, action of boiling with water and action of alkali.
2. Digestibility as indicated by hydrolysis with hydrochloric acid, diastase and pancreatin.
3. Liquifaction of starch.
4. Amylohydrolytic enzyme in stored and germinating rice.

When pure rice starch was required for experimental work it was prepared as given below:—Alkali weaker than the strength used did not remove proteinaceous matter sufficiently well while stronger solution attacked the starch grain itself.

The rice was cleaned thoroughly by repeated washings with water. It was steeped in one per cent sodium hydroxide solution and shaken for three hours with the help of hot air engine. The supernatent turbid liquid was removed and the soaked grains were washed with water and ground to a fine condition in a mortar and passed through muslin cloth. The material was treated with

half per cent sodium hydroxide for two days. The alkaline liquid was removed and a small quantity of dilute hydrochloric acid was added to the settled portion to neutralize the alkali. The starch was washed free of chlorides, drained and dried in air. The starch was freed from oil by treatment with ether and alcohol. The purified starch was found to contain 0·013 per cent nitrogen.

II. Some physical properties of rice starch

Size of the rice starch grain

Starch was prepared by the process already described from three varieties—Ambemohor, Halwar and Mahadi, both from the old (1928 harvest) and the new (1930 harvest) samples. The starch was put in water, transferred to a clear slide and examined under a high power with one-twelfth immersion lense. About 200 starch grains of each type were measured with the help of an occular and stage scale. A large number of starch grains from each group gave the following measurements.

Table II

Size of starch grains in mm.

Variety and age	Size in mm.
New Ambemohor	0·0005
Old Ambemohor	0·0005
New Halwar	0·00063
Old Halwar	0·00063
New Mahadi	0·00075
Old Mahadi	0·00075

The new and old samples do not show any difference in size but different varieties do show difference in the size of starch grains. Ambemohor is a fine variety and contains the small-sized grains while Halwar and Mahadi which are coarse and are used for bread-making have comparatively large-sized grains. In general appearance the starch grains of all the varieties are alike and have the usual characteristic polygonal shape.

Behaviour towards methylene blue and iodine

If starches are treated or ground with dye stuffs they exhibit different shades and according to Huberer and Venkatraman [1926 and 1927] it forms a reliable method of distinguishing various starches under the microscope. The rice starches were subjected to these colour tests. A small quantity of starch was made into a thin paste and about two to three drops of half a per cent methylene-blue solution were added to it. After about half an hour a portion of it was transferred to a slide and was viewed under the microscope. No difference in the starch of different varieties or age in rice could be marked. Staining with dilute iodine solution also could not show any difference.

Effects produced by boiling water

Rice grains of different varieties and of different ages, crushed and uncrushed were boiled with water for various periods and observations were made with regard to (1) swelling, (2) softening, (3) breaking of the grain coat and (4) attack on the internal contents. Each of these effects was judged separately by qualitative measurements and these were added and percentage effects were noted. The swelling and softening of the grain are less important than the breaking of the grain-coat and the attack on the internal contents and hence complete swelling and complete softening carried two marks each while complete breaking of the grain-coat and full attack on the internal contents carried three marks each. Thus a complete all-round effect carried ten marks. The following table will show how marks were given and how the total effect was calculated.

TABLE III

Marks under each heading and total marks

Time in minutes	Swelling. Marks out of two	Softening. Marks out of two	Breaking of grain-coat. Marks out of three	Attack on internal contents (Marks out of three)	Total obtained out of 10 obtainable	Total effect
						Per cent
5 minutes .	0·25	0·25	0·25	0·25	1	10
10 minutes .	1	0·5	0·5	0·5	2·5	25

The explanation of the table is as follows:—The rice grains boiled for five minutes got 0·25 marks out of two for swelling, this means the swelling produced was one-eighth of complete swelling. The marks under other items were similarly given. The total of these is one as given in column six. This one is out of ten marks obtainable. The total effect of 5 minutes' boiling is therefore 10 per cent of what the total effect would have been if swelling, softening etc. had all taken place to the fullest extent. The method is arbitrary but some method had to be adopted to give qualitative measurements to show definitely the differences in the effects produced.

The following table gives comparatively the total effect produced by boiling the rice grains of five different varieties for varying periods. In these varieties both the old and the new grains were subjected to the test of boiling with water.

TABLE IV

Total effect produced by boiling rice grains with water for different periods expressed in percentage

Time	Ambemohor		Kolamba		Patni		Halwar		Mahadi	
	New	Old	New	Old	New	Old	New	Old	New	Old
Minutes	Per cent		Per cent		Per cent		Per cent		Per cent	
5 . .	2·5	20	..	20	..	10	0	5	0	2·5
10 . .	5	60	..	60	..	22·5	2·5	8	2·5	3·5
15 . .	30	75	..	75	..	55	7·5	12·5	5	10
30 . .	65	100	..	100	..	65	57·5	57·5	30	50

The effect produced by boiling the rice grains is not equally quick on all the varieties. Ambemohor and Kolamba are the quickest and Mahadi the slowest of the five varieties tried. Halwar is nearly the same as Mahadi with regard to the effects produced. It is these two varieties which show greater resistance to the attack of water than others, and are specially used for bread-making, perhaps because they are not quite so suitable for preparing boiled rice.

The old rice grains are affected more quickly than new rice grains of the same variety, indicating that some change takes place in the grain during storage and it is of such a character that the grain becomes liable to quicker effect of boiling water.

In the case of Ambemohor variety the action of boiling with water was tried on crushed seed also. The action, as expected, is quicker on the crushed than on the uncrushed grains as shown below.

TABLE V

Total effect of boiling with water

Condition	Boiled for 5 minutes	Boiled for 10 minutes	Boiled for 15 minutes	Boiled for 30 minutes
	Per cent	Per cent	Per cent	Per cent
Crushed grains . . .	25	65	80	100
Uncrushed grains . . .	20	60	75	100

Effect of boiling with sodium bicarbonate.—Boiling with half a per cent of sodium bicarbonate solution showed the same differences for the grains of different varieties as boiling with water. In the case of Ambemohor both old and new grains were boiled with half a per cent sodium bicarbonate solution. The comparative figures showing the total effect are given in the following table.

TABLE VI

Total effect of boiling rice grains with half a per cent sodium bicarbonate

Period in minutes	Ambemohor		Kolamba	Patni	Halwar	Mahadi
	New	Old	Old	Old	Old	Old
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
5 . . .	2·5	35	35	10	7·5	5
10 . . .	7·5	65	65	30	15	12·5
15 . . .	35	80	80	60	50	20
30 . . .	75	100	100	80	65	60

Action of caustic soda on the rice grains.—Warth and Darabsett [1914] studied the disintegration of rice grains with the object of classifying the different varieties of rice according to the resistance they offered to the alkali

treatment. They came to the conclusion that on this basis rices could be classified. The disintegration roughly corresponded with the quality of the starch. Rice grains which contained much of liquifiable starch, disintegrated easily. The coarse variety grains were not so easily attacked as the grains of the finer varieties. Warth and Darabsett studied the disintegration with the help of microphotographs of the attacked grains and also from the general breaking down of the grains. For the purpose of this paper the comparative general breaking down of the grains was studied. It included observations on the following:—(1) swelling of the grain, (2) decolourisation, (3) transparency, (4) breaking of the seed-coat, (5) softening of the grain and (6) effect on the internal contents. Marks were assigned for comparison. Complete action in each of the first three items carried one mark, complete action in each of the next two items carried two marks and the last item carried three marks for its complete action. The total marks for all the complete actions were ten and the percentage of the total of all the effects in each case was calculated as explained under the effects produced by boiling with water.

The first effect observed of the treatment with alkali, is the decolourisation of the grains and softening them. There is a general swelling followed by the breaking down of the coat of grain. Ultimately the starch is attacked which becomes gelatinised and transparent.

The strengths of the caustic soda used were (1) two per cent, (2) three per cent, (3) four per cent and (4) five per cent, and the periods for which the grains were subjected to the action of caustic soda were (1) one hour, (2) two hours, (3) three hours, (4) four hours and (5) twenty-four hours. Ten grains (two years old) were studied under each treatment the quantity of the alkali being 10 c.c. for every strength.

The table gives the total effect produced on rice grains by treatment with different strengths of caustic soda.

TABLE VII

Total effect on rice grains produced by caustic soda treatments expressed in per cent

Variety	Period of treatment				
	One hour	Two hours	Three hours	Four hours	Twenty-four hours
			Two per cent caustic soda solution		
Ambemohor .	10	12	17	60	..
Kolamba . .	10	12	17	60	..
Patni . .	7	7	7	30	..
Halwar . .	7	7	7	17	..
Mahadi . .	5	5	7	15	..
			Three per cent caustic soda solution		
Ambemohor .	15	20	60	70	97
Kolamba . .	15	20	60	70	97
Patni . .	12	15	60	70	97
Halwar . .	10	12	17	25	95
Mahadi . .	10	10	15	22	80
			Four per cent caustic soda solution		
Ambemohor .	55	82	100	100	100
Kolamba . .	55	82	100	100	100
Patni . .	12	32	55	70	100
Halwar . .	7	25	45	65	92
Mahadi . .	5	15	35	37	85
			Five per cent caustic soda solution		
Ambemohor .	60	85	100	100	100
Kolamba . .	60	85	100	100	100
Patni . .	15	62	70	80	100
Halwar . .	7	42	47	70	92
Mahadi . .	7	30	40	62	87

Caustic soda acts on rice grain breaking the coat, softening the internal contents and gelatinising the starch, its action increases with the strength of the solution and with the length of the period for which the caustic soda solution acts on the grain. All the varieties are not equally attacked. There is a varietal difference shown in the resistance offered by the grains to the attack of the alkali. Ambemohor and Kolamba are the easiest attacked out of the five varieties tried and the last two, namely the Halwar and the Mahadi, are the most resistant. This again shows that it is the fine varieties which show less resistance in breaking down or in disintegration than the coarse varieties used ordinarily for bread-making. Ambemohor breaks down to the extent of 60 per cent in four hours even with such a weak solution as two per cent caustic soda while the Mahadi variety requires five per cent solution to bring about that effect in the same time.

The action of caustic soda was used to see if rice grains of different ages showed difference in resistance. Rice grains of three varieties were tried with three per cent solution for varying periods. The results show that the older the grain the less is its resistance to the attack of caustic soda. This is shown by the figures in the following table.

TABLE VIII

Total effect on new and old grains by the action of three per cent caustic soda expressed in per cent

Period	Ambemohor		Halwar		Mahadi	
	New	Old	New	Old	New	Old
Hour						
1 . . .	10	15	5	10	5	10
2 . . .	12	20	5	12	5	10
3 . . .	40	60	12	17	12	15
4 . . .	60	70	17	25	15	22
24 . . .	95	97	85	90	70	80

Rice grains of the Ambemohor variety were subjected to the treatment of three per cent caustic soda at two different temperatures—27°C. and 37°C.—under crushed and uncrushed conditions and also under polished and unpo ished conditions. Difference in condition always gave difference in the resistance of the grain to the attack of the alkali. Higher temperature produced quicker reaction than lower temperature. Unpolished grains showed greater resistance than polished grains and uncrushed grains showed greater resistance than crushed grains.

Conclusions

The following conclusions can be drawn from the results of the experiments given in this section. The results, it must be admitted, are worked out after the fashion of score-card judging. They are intended to give a comparative idea about the disintegration of rice grains under different conditions.

(1) Boiling with water affects the rice grain. The longer the boiling the greater is the effect.

(2) The effect of boiling with water is greater on old rice grains than on new grains.

(3) Boiling with water does not affect the rice grains of all varieties equally. Those that are generally used for bread-making show greater resistance than those used for boiled rice.

(4) Crushed grains are naturally attacked more quickly than uncrushed ones.

Similar conclusions can be drawn from the experiments done with caustic soda treatment.

(5) Varietal and age differences are shown by treating rice grains with caustic soda. The caustic soda attacks grains of all the varieties and ages.

(6) The longer the period of attack and the higher the strength of the caustic soda solution the greater is the effect produced.

(7) Rice varieties for bread-making are more resistant than fine varieties used for boiled rice.

(8) New rice grains show greater resistance than old grains.

(9) Crushed and polished condition as also higher temperature tend to increase the effect on the grain.

III. Hydrolysis of rice starch with hydrochloric acid and diastase and pancreatin

As the chief object of the investigation was to study the differences in the digestibility of rice of different varieties and different ages, experiments were arranged to see the effects of hydrochloric acid on starches and flours, and of diastase and pancreatin on flours. Many workers have studied the hydrolysing action of hydrochloric acid and enzymes on starches of various food grains. Stone [1896] carried on experiments to study "the degree of susceptibility of different starches to the action of different enzymes". He established an order of decreasing susceptibility of various starches. He also mentions Lenberg [1877] and Hammersten [1883]. Each one of these gave his own order of susceptibility for saccharification. Rockwood [1910] studied the action of pancreatin, ptylin and diastase on the starch from bleached and unbleached wheat flours and he found that the reducing sugars were formed at equal speed in both the cases as tested by starch-iodine reaction. Langworthy and Deuel [1920] carried on interesting work on the digestibility of certain starches on human subjects. A good deal of work has been done by several workers like Brown and Morris [1895], Ling and Baker [1895], Lintner [1895] and others,

Action of hydrochloric acid on starches

By way of trial hydrochloric acid of 2·5, 25, 50, 75, and 100 per cent was used but it was observed that 100 and 75 per cent hydrochloric acid was too quick in its action to show differences in its actions on different starches while any strength lower than 25 per cent proved to be too slow. Fifty per cent strength was found to be the most suitable for measuring differences and hence that strength was used. Starches from three different varieties and of different ages were experimented upon.

In each case one grm. of starch was made into a paste with 5 c.c. of water. 25 c. c. of 50 per cent hydrochloric acid was then added and the solution thoroughly stirred to get a uniform mixture. The hydrolysis was allowed to proceed for a fixed period at a temperature of 27°C. At the end of the period the acid was neutralised with sodium carbonate and the whole volume made up to 200 c. c. The solution was then filtered through dry filter paper and titrated against Benedict's [1910-11] solution which was standardised. The reducing sugars were estimated as dextrose and the figures for dextrose were multiplied by 0·93 (conversion factor for starch) to get starch. Thus the amount of starch digested was obtained from which the per cent digested was calculated. Each set had a duplicate. The results of the duplicates agreed with each other and hence there was no need to take averages. The hydrolysis was carried on in one set for 24 hours and in another for 72 hours. The varieties used were (1) Ambemohor, (2) Halwar and (3) Mahadi, both new and two years old.

TABLE IX

Percentage of starch hydrolysed

Variety	Age	Hydrolysis	
		24 hours	72 hours
		per cent	per cent
Ambemohor	New . .	34·29	64·62
	2 year old .	46·80	76·14
Halwar	New . .	33·84	63·90
	2 year old .	46·80	76·14
Mahadi	New . .	34·29	63·45
	2 year old .	45·18	74·97

The figures show that the longer the period of attack the greater is the amount of hydrolysis. Both for 24 hours and 72 hours digestion the old starches are more easily attacked than the new ones. There is, however, very little difference shown by different varieties with regard to the hydrolysis by 50 per cent hydrochloric acid.

Action of hydrochloric acid on rice flour

It is after all the rice flour and not the purified starch that is used as food and hence the hydrolysing action of hydrochloric acid, 50 per cent. in strength, was tried on flours. Rices of the same three varieties and the same two different ages were used. The polished rice grains of each type were ground to a fine powder. Half a grm. of the flour was made into a paste with 10 c.c. water and to this 10 c.c. of 50 per cent. hydrochloric acid was added and well stirred. The hydrolysis was carried on at a temperature of 27°C. for 48 hours. The acid was neutralised with sodium carbonate and the estimation of reducing sugars and calculation of digested starch was done as in the previous experiments.

The results obtained are given in the following table. They are of hydrolysed starch per 100 grms. of the flour and not per 100 grms. of starch in the flour.

TABLE X

Per cent of hydrolysed starch with 10 c.c. of 50 per cent hydrochloric acid at 27°C. in 48 hours

Variety	Year of harvest	Starch hydrolysed
		per cent
Ambemohor		
New	1930	7·74
Two year old	1928	8·46
Halwar		
New	1930	6·30
Two year old	1928	6·66
Mahadi		
New	1930	5·76
Two year old	1928	7·20

The flours from two year old rices are more easily attacked than those of new rices.

The same rice samples (those of 1928 harvest and 1930 harvest) were stored for a period of two years more, that is to the end of 1932 and were subjected to the action of 50 per cent. hydrochloric acid exactly in the same manner as in the previous experiments.

Rice grains harvested at the end of 1928 were four years old and those harvested at the end of 1930 were two years old at the time they were experimented upon. The above table gives the difference between new and two-year old rices while the following table gives the difference between two-year old and four-year old rices.

TABLE XI

Per cent of hydrolysed starch by 10 c.c. of 50 per cent hydrochloric acid at 27° C. in 48 hours

Variety	Age	Harvested	Starch hydrolysed
		Year	Per cent
Ambemohor	2 year old .	1930	12·82
	4 year old .	1928	14·18
Halwar	2 year old .	1930	9·86
	4 year old .	1928	10·52
Mahadi	2 year old .	1930	9·10
	4 year old .	1928	10·14

Flour from four-year old rice is more easily broken down than the flour from two-year old rice. These, however, were samples from different stocks and to get a better idea, samples from the same stock should be compared. Taking the same sample of 1928 harvest kept for two years and hydrolysed in 1930 and for four years and hydrolysed in 1932 we can put the figures as follows:—

TABLE XII

Per cent of hydrolysed starch by 10 c.c. of 50 per cent hydrochloric acid at 27°C. in 48 hours

Variety	The same stock	
	Two year old (Harvested in 1928 and hydrolysed in 1930)	Four year old (Harvested in 1928 and hydrolysed in 1932)
	Per cent	Per cent
Ambemohor	8·46	14·18
Halwar	6·66	10·52
Mahadi	7·20	10·14

These figures show clearly the change produced during a period of two years in storage; with increasing age the rice becomes easier for attack by chemical and other agencies.

Action of diastase absolute

Diastase is an enzyme which acts on starch breaking it down to reducing sugars. It is, therefore, a good reagent to measure the differences between varieties and ages of rice. The strength of diastase used was 0·1 per cent of Merck's diastase absolute. The action was tried for one-hour-and-a-half at a constant temperature of 37°C. The object in selecting 37°C. temperature was to try the digestion of starches by the enzyme at body temperature. Blank experiment with diastase only was carried on to ascertain if any quantity of reducing sugars was produced by the enzyme and to make necessary correction in the results obtained, but the diastase used did not give any reducing sugars. The strength of the diastase activity was compared by experimental trials and it was found that one c.c. of one per cent diastase was equivalent to 0·75 c.c. of concentrated hydrochloric acid (sp. gr. 1·16) under the same condition of temperature, dilution, etc.

Half a grm. of the flour was made into a thin paste with 10 c.c. water in a large test-tube and was gelatinised by keeping it in a boiling water-bath for three minutes. It was then cooled to room temperature and 100 c.c. of diastase solution of required strength was added to the gelatinised starch. To prevent fermentative and other changes a few drops of toluene were added to

the mixture. The tube was after that immediately put into a water-bath kept at a constant temperature of 37°C. The reaction was allowed to continue for the required period. The enzyme was killed by adding a few drops of 10 per cent caustic soda and by immersing the test tube in boiling water for about 10 minutes. The contents of the tube were cooled to room temperature and made to a known volume in a graduated flask. The solution was filtered through dry filter paper and titrated against standardised Benedict's solution for the estimation of reducing sugars formed. The figure for reducing sugars was multiplied by 0·93 to get the amount of starch and the percentage of starch hydrolysed was calculated. A number of such tubes necessary for the experiment were handled in one lot. Each trial had a duplicate and the duplicates agreed with each other.

As in the case of hydrochloric acid three varieties of rice—Ambemohor, Halwar and Mahadi, both new and two-year old were tried, and the results obtained with 0·1 per cent diastase solution for one hour-and-a-half at 37°C. are given below.

TABLE XIII

Per cent of hydrolysed starch with 10 c.c. of 0·1 per cent diastase solution at 37° C. for 1·5 hours

Variety	Year of harvest	Starch hydrolysed
	End of	Per cent
Ambemohor		
New	1930	21·60
Two year old	1928	31·68
Halwar		
New	1930	8·70
Two year old	1928	16·12
Mahadi		
New	1930	18·00
Two year old	1928	31·14

The results also indicate that there are differences in the starches of different varieties with regard to the action of diastase. Flours of old grain are attacked more easily than those of new grains.

The same rice samples were stored for a period of two years more and were then subjected to the action of diastase as above to get the difference between two-year old and four-year old rices.

TABLE XIV

Per cent of hydrolysed starch with 10 c.c. of 0·1 per cent diastase solution at 37° C for 1·5 hours

Variety	Age	Harvest	Starch hydrolysed
			Per cent
Ambemohor	2 year old .	1930	24·70
	4 year old .	1928	35·83
Halwar	2 year old .	1930	11·90
	4 year old .	1928	17·16
Mahadi	2 year old .	1930	18·80
	4 year old .	1928	35·40

The two-year old and four-year old samples show a distinct difference. The samples of two-year old and four-year old as compared in the above table do not belong to the same stock. Figures from Tables XIII and XIV can be taken and arranged as below to show the difference between the two-year old and four-year old rices belonging to the same original stock for proper comparison.

TABLE XV

Per cent of hydrolysed starch with 10 c.c. 0·1 per cent diastase solution at 37° C. for 1·5 hours

Variety	Two year old	Four year old
	Per cent	Per cent
Ambemohor	31·68	35·83
Halwar	16·12	17·16
Mahadi	31·14	35·40

This clearly indicates the change produced during a period of two years in storage. In the above experiments samples from one and the same stock were taken and hydrolysed at various times to see the effect produced during storage. Another experiment was arranged in which rices belonging to the same variety and strain but harvested in different years were subjected to the action of hydrolysis at one and the same time. For this purpose only one variety was taken and that was Ambemohor. Grains from the harvests of 1928, 1930, 1931 and 1932 were taken and subjected to hydrolysis in the beginning of 1933 so that there was a new sample collected at the end of 1932, one-year old sample, two-year old sample and four-year old sample. Only one period of reaction was tried. The material consisted of finely ground rices of different ages. 20 c.c. of concentrated hydrochloric acid was used with one set and 20 c.c. of one per cent diastase solution was used with another set. Period of action was 24 hours kept at 27°C. The estimations were made as in previous calculations, the percentages being determined on flours.

TABLE XVI

Per cent of hydrolysed starch in 24 hours at 27° C.

Year of harvest	Age	With concentrated hydrochloric acid	With one per cent diastase
		Per cent	Per cent
1928	Four year old .	58·99	49·40
1930	Two year old .	50·12	45·45
1931	One year old .	47·61	43·64
1932	New . .	46·50	41·17

The new rice shows the least hydrolysis. As the age of the grain increases the percentage of hydrolysis also increases. The results obtained with hydrochloric acid and with diastase are quite parallel.

Action of pancreatin

It has been shown that there is a clear difference between starches from old and new samples of rice when they are acted upon by hydrochloric acid and by diastase. Pancreatin is another enzyme which acts on the starch and hence the action of this also was tried to see if it could distinguish between the old and the new rices. The same varieties and the same ages of rices were done in the same manner as in the previous experiments.

One-fourth grm. of starch was taken and was gelatinised in test tubes with 10 c.c. water. After cooling to the room temperature 10 c.c. of different strengths of pancreatin were added and the reaction was allowed to proceed for a definite period at 37°C. A few drops of toluene were added to prevent bacterial action. It was observed that pancreatin was not easily soluble in water and hence it was shaken vigorously for a minute and a measured portion was pipetted out as required. After the digestion period was over the test-tubes with starch and pancreatin were put in a boiling water-bath for 15 minutes to kill the pancreatin. The solutions were made to a known volume and filtered through dry filter paper. After titration against Benedict's standardised solution the dextrose figures were multiplied by 0·93 to get starch. The percentage figures were then calculated.

The strengths of pancreatin taken were 10 c.c. of 0·5 per cent and 10 c.c. of one per cent and the periods of digestion were two hours and three hours. In each case duplicates were done and they agreed with each other.

TABLE XVII

Per cent of starch hydrolysed with pancreatin

Variety	Half per cent pancreatin		One per cent pancreatin	
	Two hours	Three hours	Two hours	Three hours
	Per cent	Per cent	Per cent	Per cent
Ambemohor				
New	Traces	Traces	4·32	4·93
2 year old	29·52	36·00	32·24	43·36
Halwar				
New	13·68	14·76	20·16	32·24
2 year old	24·12	26.28	30·60	44·64
Mahadi				
New	25·92	36·00	34·56	41·76
2 year old	33·12	45·56	44·28	49·68

It will be seen that there is a distinct difference in the susceptibility of the starches of new and old rices towards pancreatin. The starch prepared from old rices undergoes greater amount of hydrolysis than that prepared from new ones. The effect of prolonging the period of reaction or increasing the strength of the enzyme is to increase the amount of action. In all cases the difference between the old and the new rices persists. One striking feature in the above experiments is that the starch from new Ambemohor is practically not acted upon by 0·5 per cent pancreatin and only slightly acted upon, as compared to other starches, by one per cent pancreatin. In the previous experiments Ambemohor starch was the quickest to be acted upon by hydrochloric acid and also by diastase. With pancreation, however, that position does not remain the same Especially new Ambemohor seems to resist the action of pancreatin very much.

Conclusions

Rices of three varieties—Ambemohor, Halwar and Mahadi—both old and new were subjected to the hydrolysing action of hydrochloric acid, diastase and pancreatin to ascertain if the amount of action produced showed any difference in the old and new rices.

The hydrolysing action of different strengths of hydrochloric acid, diastase and pancreatin acting for varying periods was more on old rices than on new ones whether used as flour or as prepared starch.

If the three rices are hydrolysed at one time and again after two years a distinct difference in the percentage of hydrolysis is seen, the second trial (after two years) giving greater hydrolysis than the first. This indicates that as rices grow old they become more susceptible to the action of hydrolysis.

If the rices of the same strain and variety but harvested in different years are subjected to hydrolysis, a distinct difference is seen. The oldest rices are attacked the most and the new ones the least.

With hydrochloric acid and diastase Ambemohor starch is hydrolysed more quickly than that of the other varieties used for bread-making. This, however, does not hold good with pancreatin.

In all cases the hydrolysis increased with increasing strength of the reagents and with increase in the period of action.

All the reactions, therefore, point to the conclusion that old rices are easier to digest than the new ones.

IV. Liquefaetion of starch in new and old rices

Introductory

The general belief that old rices are more easily digested than the new ones has been confirmed by the results obtained so far both qualitatively and quantitatively. Now the question arises as to why there is such difference in the behaviour of the two. There are two probable answers to this question, one of which is that the starch in the old rices may break down into more easily digestible portions during the resting period, and the other is that during the

process of cooking the starch of the old rices may undergo greater liquefaction than that of the new ones, so that it is better attacked by the digestive enzymes of the human system.

As regards the first possibility it has been found out that the resting seeds do contain an Amylo-hydrolytic-enzyme (proved further on) and if this is active during the resting period, it may attack the starch of the grains and thus turn it into simpler units. This enzymic breaking-down may be further helped by a physical disintegration of the grain, and these two processes may make the old rices more susceptible to the action of the digestive ferments. This section, however, aims at studying the liquefaction of starch at different temperatures, with the object of finding out differences in the starch prepared from new and old rices if there be any, and thus to account for the difference in the digestibility of the two to a certain extent. That the cooking quality of starch is closely co-related with the extent of its liquefaction has long been shown by the work of Warth and Darabsett [1914] and needs no further explanation.

It is however interesting to note at this stage what happens to the starch during the process of cooking. The most important change that takes place is that the structure of the grain changes to a great extent both internally and externally. The granules break down and rush out of the ruptured cell-walls. This gives the characteristic jelly-like appearance to the whole swollen mass, when probably the starch in the grain is said to be gelatinised. In the process of gelatinisation the starch is to a great extent liquefied and it is this liquefied starch that is most easily attacked by the digestive enzymes.

Fofanow [1911] carried on some experiments on human subjects with raw starch of wheat, oat, rice, potato and he came to the conclusion that all of them were completely assimilated but that potato starch was less digestible than others. He further said in his work that "numerous artificial experiments have proved that the raw starches are more slowly attacked than those that have been cooked, by the digestive ferments".

Langworthy and Deuel [1920] who studied the digestion of raw wheat, corn, and potato starches came to the conclusion that all of them were completely assimilated though of course the potato starch caused some physiological disturbance. They further say that "Diastase has only a feeble action on uncooked starch".

From the work of the above workers it may be seen how cooking facilitates the digestion of starch and what role it plays in the chemical and physical changes that the starch undergoes during this process. The only point with which we are concerned is the liquefaction of the starch during the process of cooking.

Experimental

Two grms. of rice in a finely powdered condition was mixed with 25 c.c. of distilled water and was kept at fixed temperatures for one hour and a half, the temperatures being regulated in a water-bath. Thus all the starch that could be liquefied at that particular temperature was present in the solution. The contents of the test-tubes were then hydrolysed at 27°C. by 5 c.c. of one per cent diastase absolute, for two hours. The solution was then made to a known

volume after killing the enzyme with two to three drops of strong caustic soda. The solution was then filtered and 50 c.c. of it was subsequently hydrolysed by 15 c.c. of hydrochloric acid by keeping the containers in a boiling water-bath for two hours. The contents were further made to a known volume, after neutralising the excess of the acid with sodium carbonate, and the reducing sugars were estimated as dextrose by titration against standardised Benedict's solution. From this the amount of starch liquefied at that particular temperature was calculated. Only three varieties of rices were studied and these belonged to 1930 and 1928 harvests.

The following temperatures were tried for the liquefaction:—(1) 55°C. (2) 65°C. (3) 75°C. and (4) 95°C.

Commercial diastase does not act on crude starch. This means that in the present trials it is only the liquefied starch that is attacked by the commercial diastase used, at the different temperatures tried. The following are the results obtained in the present trials.

TABLE XVIII

Per cent. liquefaction of starch in the new and old rices

Variety	Liquefaction			
	55°C.	65°C.	75°C.	95°C.
	Per cent	Per cent	Per cent	Per cent
New Ambemohor	7·06	12·38	29·76	46·50
Old Ambemohor	7·83	15·49	37·20	49·46
New Patni	6·88	14·88	25·66	45·08
Old Patni	7·44	21·24	29·74	58·12
New Mahadi	6·88	10·04	20·27	40·21
Old Mahadi	8·74	11·34	24·74	49·46

The figures of the above table show that (1) the percentage liquefaction in general increases along with the temperature and is higher with higher temperature. (2) There is a significant difference in the behaviour of new and old rices so far as the liquefaction of the starch in them is concerned. The starch in the old rices undergoes greater amount of liquefaction than that in the new ones. This is perhaps the reason why old rices are more easily digested than the new ones.

V. Study of the amylase enzyme developed in the resting and germinating seede of rice

Introductory

It has been noted that during the process of "keeping" the rice seeds undergo a sort of disintegration and that both the grain and the starch are comparatively easily acted upon by diastase, pancreatin and hydrochloric acid. It is to be seen whether this breaking down, and easy susceptibility to the action of hydrolysing agents is an enzymic process or is due to any physical disintegration of both the grain and the starch during the course of storing time. With this object it was decided to study the amylase enzymes that are developed in the resting and germinating seeds of rice with a view to find out the difference, if there is any, in the behaviour of the two and also to compare the activities of the two with that of standardised solution of diastase absolute (obtained from E. Merck), under the same conditions of temperature and dilution.

Brown and Morris [1895] are of opinion that the "Saccharifying ferment" is entirely a product of germination and that ungerminated barley does not contain any starch-transforming body. In the same paper however they quote Kjeldahl's opinion on this point (Resume De Comptes Rendus—1875—I—129) which runs as follows:—

"He has shown the existence of an active amylohydrolytic enzyme in ungerminated barley and though this has a considerable saccharifying power upon soluble starch yet it has scarcely any influence in liquefying starch-paste." The enzyme of the resting seeds is thus differentiated from that of the germinated seeds in not having the power of liquefying starch.

It will thus be seen that an amount of work of a similar nature has been done in the past, though not on the rice seed. The following experiments were arranged to see whether amylohydrolytic action goes on very slowly in the resting seed making the seed more and more susceptible to the attack when favourable conditions occur. In this connection the amount of reducing sugars formed by the action of the enzyme alone has been taken as a measure of the activity of the enzyme. Trials with germinating seed also were taken for comparison.

Experimental

For the study of the enzymes of the resting and germinating rice seeds, the experiment was laid out into nine different lots and each lot was analysed separately for dextrose, the amount and formation of which was taken as a measure of the activity of the enzyme. In the case of all the lots duplicate trials were made and the dextrose formed as a result of the action of the enzyme was estimated by Benedict's method. In the case of the resting seeds those of previous year's harvest were used and in the case of germinated seeds those that were in the soil for 96 hours (4 days) were used. All natural conditions regarding the amount of moisture, uniform sowing, etc. were given to the seeds and the germination was complete by the 4th day, when the seeds had put forward

both the radicle and the plumule. The average length of the plumule was about 1·25 cm. and that of the radicle was nearly one cm. The plumule was slightly green in colour.

Seeds of the above description were used in the lot of "germinated seeds" and before preparing an extract with distilled water, they were completely freed of all the adhering soil and the portions of shoots and roots. The following were the lots made in the present experiment:—

Lot I.—Blank—*i.e.* Extract of resting seeds.

Lot II.—Blank—*i.e.* Extract of germinated seeds.

Lot III.—Blank—*i.e.* Extract of 0·5 per cent. diastase absolute.

Lot IV.—Ungelatinised starch *plus* extract of resting seeds.

Lot V.—Ungelatinised starch *plus* extract of germinated seeds.

Lot VI.—Ungelatinised starch *plus* extract of 0·5 per cent diastase absolute.

Lot VII.—Gelatinised starch *plus* extract of resting seeds.

Lot VIII.—Gelatinised starch *plus* extract of germinated seeds.

Lot IX.—Gelatinised starch *plus* extract of 0·5 per cent diastase absolute.

Preparation of the extracts

In the case of resting seeds those of two-year old harvest were used, and in the case of germinated seeds one-year old seeds which were in the soil for 4 days were used. The germinated seeds were cleaned of all their adhering dirt and the portions of the shoots and roots, and then the extract of the same was made. In both the lots 30 grms. of seeds (germinated and resting) were used and they were extracted with 100 c.c. of distilled water for 24 hours, after shaking the same on a machine run by hot air, for 5 hours. During extraction a few c.c. of toluene were added to prevent fermentation and other changes. These extracts were used for the hydrolysis of the rice starch. As said before the experiment was laid out into 9 different lots and the hydrolysis was carried on at the temperature of 27°C. under the same conditions of dilution, etc. In the case of diastase solution 20 c.c. of 0·5 per cent strength was used and in the case of all other extracts the same volume *i.e.* 20 c.c. was used for the hydrolysis. The amount of starch used for the lots was 0·25 grms., and for lots with gelatinised starch this quantity was treated with a few c.c. of water and was subsequently heated on a sand-bath. The hydrolysis was allowed to proceed for 24 hours and at the end of this period the action was checked by the addition of a few drops

of strong alkali (sodium hydroxide) and subsequently boiling the mixtures on a sand-bath for a few minutes. During the period of reaction the fermentative changes were checked by the addition of a few drops of toluene to the reacting mixtures. After making the volume to 100 c.c. the mixtures were filtered through a dry filter paper and were subsequently titrated against standardised Benedict's solution. The activity of the enzymes has been expressed in terms of mgs. of dextrose formed from equal quantities of starch under the same conditions of temperature, dilution of the extract (strength of the extracts), etc. The following are the results obtained in these trials:—

TABLE XIX

Amount and extent of hydrolysis effected by the enzyme of the resting seeds and that of the germinated seeds, under similar conditions in terms of mgs. of dextrose formed from gelatinised and ungelatinised rice starch

Lot No.	Details of the lots	Dextrose formed	Duplicate
		Mgs.	Mgs.
I	Blank with extract of resting seeds . . .	39·24	39·24
II	Blank with extract of germinated seeds . .	44·44	44·44
III	Blank with extract of 0·5 per cent diastase absolute	0·0	0·0
IV	Ungelatinised starch *plus* extract of resting seeds .	64·51	64·51
V	Ungelatinised starch *plus* extract of germinated seeds.	68·68	69·44
VI	Ungelatinised starch *plus* extract of 0·5 per cent diastase absolute.	Traces	Traces
VII	Gelatinised starch *plus* extract of resting seeds .	102·5	105·26
VIII	Gelatinised starch *plus* extract of germinated seeds	312·5	307·6
IX	Gelatinised starch *plus* extract of 0·5 per cent diastase absolute.	222·2	222·2

From the above table we find that the extracts of both the resting and germinating seeds show the same amount of dextrose and that the extract of germinated seeds shows a little more of the same than the extract of resting seeds. Thus for the former we have 44·44 mgs. and for the latter we have 39·24 mgs.

of dextrose. This means that the resting seeds do contain some amount of dextrose. In the case of 0·5 per cent extract of diastase absolute, a blank experiment with the same gave no test for reducing sugars. Going now to the fourth lot we find that 64·5 mgs. of dextrose have been formed from 0·5 grms. of rice starch by the action of 20 c.c. of extract of resting seeds for a period of 24 hours. This proves the existence of an enzyme in the resting seeds, which is capable of attacking ungelatinised starch to some extent. By comparing the figure obtained for the first lot *i.e.* (for the blank with extract of resting seeds—39·24 mgs.) with that of the fourth one we find that the latter gives us an increase of nearly 25 mgs. which is clearly due to the action of the enzyme of the resting seed on ungelatinised starch. Going now to the fifth lot we find that the figure for the dextrose here is slightly higher than that for the previous one, the increase being a little more than four mgs. This increase is, therefore, due to the slightly greater activity of the enzyme of the germinated seeds and this activity may be probably due to the better conditions of temperature and moisture that are given to the germinating seeds.

It had already been pointed out in the Introductory part of this section that Brown and Morris [1895] are of opinion that the resting seeds do not contain any "starch-transforming body" and that "it is entirely a product of germination". However in the light of the results obtained in the present work it can be safely said that though slightly less active than the enzyme of the germinating rice seeds the resting seeds do contain such a starch-transforming body and that during the process of germination this body increases in its activity. These results are supported by the work of Kjeldahl [1880] who has actually proved the existence of "An active amylo-hydrolytic enzyme in ungerminated barley." Brown and Morris are of opinion that though this enzyme as shown to be present in ungerminated barley has a considerable saccharifying power yet it has "scarcely any influence in liquefying starch paste." Though in the present paper the actual liquefying power has not been studied yet it can be safely said from the results obtained that the enzyme of the resting seeds does act on ungelatinised rice starch and it could not be so if it had no liquefying power. Or it may be that even this enzyme may be a complex of two or more co-enzymes one of which may be with some power in liquefying the starch to some extent which is then attacked by the other and turned into dextrose.

Going now to lot six we find that we hardly get any hydrolysis and this means that an extract of diastase absolute (0·5 per cent) is not able to act on ungelatinised starch as the other two do, and slight traces of dextrose are formed in this case. This is supported by Brasse [1885] who says that "Commercial diastases have no effect on crude or uncooked starch, and this is due to the alteration that they undergo in the process of extraction."

Lot seven which represents the action of the extract of resting seeds on gelatinised starch, shows 102·5 mgs. of dextrose, and 105·26 mgs. as a duplicate of the same. Lot eight, which stands for the action of the extract of germinated seeds on gelatinised starch shows 312·5 mgs. of dextrose and 307·6 mgs. as its duplicate. Lot nine gives 202·2 mgs. and this stands for the action of diastase absolute (0·5 per cent) on gelatinised starch. This means that an extract of

germinating seeds is more active than that of the resting seeds. Comparing the figures of lots seven and eight with those of nine we find that an extract of diastase of 0·5 per cent strength has greater action on gelatinised starch than an extract of resting seeds (strength 30 per cent) though an extract of germinated seeds has greater action still than the above two. Similarly an extract of germinating seeds gives 210 mgs. and about 110 mgs. more of dextrose than an extract of resting seeds and an extract of diastase (0·5 per cent strength) respectively when they are allowed to act on equal quantities of rice starch. The following are some of the conclusions drawn from the results obtained in the present work:—

(1) Both the germinated and the resting seeds contain some amount of dextrose and it appears that it is formed from the action of the enzyme present in the seed. The amount present in the germinated seeds is slightly greater and this is probably due to the increased activity of the enzyme during germination.

(2) The enzyme of the resting seeds does act on ungelatinised starch, though of course the action is decidedly less when compared to that of the enzyme present in the germinating seeds. Diastase absolute however is not able to act on ungelatinised starch.

(3) The action of all the three enzymes is greater on gelatinised starch than on an equal amount of ungelatinised starch.

(4) The enzyme of the germinating seeds appears to have the greatest action on gelatinised starch; then comes the diastase and last is the enzyme of the resting seeds.

(5) The action of the extract of resting seeds on ungelatinised starch proves that it has some power of liquefying the starch, or it may be that this enzyme is a complex of two or more "co-enzymes" one of which liquefies the starch and the other turns it into dextrose.

After proving the existence of an amylo-hydrolytic enzyme in the resting seeds it is easy to understand the difference between new and old rice grains. We have already seen that this enzyme has the power of attacking both gelatinised and ungelatinised starch of the rice grain and it, therefore, appears that if the enzyme remains active during the resting period of the grains it may, to a certain extent, attack the starch that is present in it. We have seen that the starch and also the flour of the old rices undergo greater action when treated with different hydrolysing agents, than the starch and flour of new ones. This easy susceptibility of the old rices to the various treatments appears to be not merely due to a physical disintegration of the grain into simpler products, during the period of storage, but also due to a certain enzymic action during this period. We have seen that during the resting period the rice and its starch change some of their physical properties, and they are attacked to a greater extent by alkalies when treated with them. It is also noticed that old rices and their starches undergo greater disintegration when boiled with water than the new rices and the starches prepared from them.

REFERENCES

Benedict, Stanley R. (1910-11). *J. Biol. Chem.* **9**, 57.

Brasse, L. (1885). *J. Chem. Soc.* **43**.

Brown, H. T. and Morris, G. H. (1895). *J. Chem. Soc.* **67**, 709.

Fofanow (1911). (quoted by Langworthy and Deuel, *J. Biol. Chem.* **42**, 1920, p. 27).

Hammerston (1883). (quoted by W. E. Stone).

Huberer and Venkatraman, K. (1926-27). *Analyst* **51**, 351 and **52**, 37.

Kejeldahl (1875). (quoted by Brown and Morris).

Langworthy, C. F. and Deuel, H. J. (1920). *J. Biol. Chem.* **42**, 27.

Lengerg (1877). (quoted by W. E Stone).

Ling, A. R. and Baker, J. L. (1895). *J. Chem. Soc.* **67**, 702—708.

Lintner, C. J. (1895). (quoted by Brown and Morris).

Rockwood, E. W. (1910-11). *J. Biol. Chem.* **8**, 327.

Stone, W. E. (1896). *U. S. Dept. Agric. Bull* **34**.

Warth, F. J. and Darabsett, D. B. (1914). *Mem. Dept. Agric. India, Chem. Ser.* **3**, No. 5.

THE MECHANICAL ANALYSIS OF LATERITIC SOILS

2. A NOTE ON THE APPLICABILITY OF THE HYPOBROMITE METHOD

BY

JOGENDRA NATH CHAKRABORTY,

AND

ASHUTOSH SEN,

Agricultural Research Section, Department of Chemistry, Dacca University

(Received for publication on 9th October 1934)

Troell [1931] has developed a new method for the mechanical analysis of soils using sodium hypobromite. The method appears to be a promising general method and has an advantage over the popular International Soda method in that the agent (sodium hypobromite) for oxidation of organic matter in the new method, unlike that (hydrogen peroxide) in the International Soda method, is unaffected by the temperature of tropical countries.

In order to see if the hypobromite method was suitable for the mechanical analysis of Indian lateritic soils, a few such soils were analysed by this method and the results compared with those obtained by the International Soda method.

TABLE I

Percentage of clay obtained by different methods

Soil No.	3	4	5
Sodium hypobromite method	15·3	14·0	11·4
International Soda method	19·2	16·3	16·6

It is seen from above that in the case of soil Nos. 3, 4, and 5 the hypobromite method gave low clay figures, in other words, the dispersion in the case of these soils was incomplete.

In the first paper of this series [Chakraborty and Sen, 1932] it has been shown that these lateritic soils refused to disperse fully unless they were shaken for 24 hours in a suspension of pH about 10·5. In the hypobromite method shaking for half an hour is recommended with a suspension whose reaction may be slightly alkaline. To test, therefore, whether dispersion improved in the case of the hypobromite method, determinations were made employing 24 hours'

shaking and making the suspension more alkaline. The results are given in Table II below:—

TABLE II

Percentage of clay obtained by different dispersion treatments

Soil No.	International Soda method	Hypobromite method ; ½ hour shaking	Hypobromite method ; 24 hours' shaking	Hypobromite method ; 24 hours' shaking pH 10·5
3	19·2	15·3	16·7	19·1
4	16·3	14·0	15·0	17·3
5	16·6	11·4	12·9	15·2
6A . . .	..	32·8	39·8	45·7
19	..	17·0	24·0	28·9

Comparison of results given in column 4 of the above table shows that better dispersion of soils was obtained by including the standard shaking procedure extending over 24 hours in the hypobromite method. Even then the amount of clay so obtained is smaller than that given by the International Soda method. But when the suspensions were made more alkaline and shaken for 24 hours the amount of clay thus obtained is practically equal to that given by the International Soda method.

It is clear, therefore, that the dispersion of some of the Indian lateritic soils by the hypobromite method is not complete unless the following modifications are introduced:—

(1) Addition of sufficient quantity of alkali so that pH of the final suspension is 10·5.

(2) A larger period of shaking than recommended in the original method.

But the greatest objection to the hypobromite method is that it requires the use of large quantities of obnoxious and injurious chemicals like bromine and ammonia and therefore quite unfit for inclusion in routine practice.

ACKNOWLEDGMENT

Our thanks are due to the Imperial Council of Agricultural Research for the maintenance of the salary of one of us (J. N. C.).

REFERENCES

Chakraborty, J. N. and Sen, A. T. (1932). *Ind. J. Agric. Sci.* **2**, 516.

Troell, E. (1932). *J. Agric. Sci.* **21**, 476.

3. A NEW METHOD USING ALKALINE PERMANGANATE FOR OXIDATION OF ORGANIC MATTER

BY

JOGENDRA NATH CHAKRABORTY,

Agricultural Research Section, Department of Chemistry, Dacca University

(Recieved for publication on 20th October 1934)

A method termed the "Direct Soda Method" for mechanical analysis of Indian lateritic soils was developed and published by the author and Sen [1932]. The essential feature of this method consists in dispersing the soil directly by shaking with water made alkaline, pH about 10·5, with sufficient caustic soda. This method was evolved in the light of the fact that the majority of Indian lateritic soils contained little organic matter, calcium carbonate and exchangeable bases so that the pre-treatment of these soils with hydrogen peroxide and hydrochloric acid as recommended in the International method was not necessary but it was shown that the dispersion medium should have a pH about 10·5. In exceptional cases where lateritic soils contained more than 2 per cent of organic matter, it was recommended to include in the method the pre-treatment with hydrogen peroxide so as to remove humus. It may be of interest to mention here that almost simultaneously with the publication of our paper [Chakraborty and Sen, 1932] embodying these results the Imperial Bureau of Soil Science brought out their Technical Communication No. 26 [Robinson, 1933] in which it was stated that while destruction of humus was always advisable to ensure complete dispersion, results of mechanical analysis of a large number of soils including laterites indicated that where less than one per cent carbon was present the depression of the clay figure due to non-removal of the organic matter was not serious. This statement corroborated our conclusion since one per cent organic carbon might be considered as equivalent to 1·72 per cent organic matter [Read and Riddgell, 1922].

It was however felt that a method for mechanical analysis should be free from limitations and that attempt should be made to find one general method for dispersion of lateritic soils in which organic matter is present in large or small quantities. Furthermore the use of hydrogen peroxide which is costly and unstable in India should be avoided. Accordingly a search was made for a suitable agent for the oxidation of organic matter. Sodium hypobromite as recommended by Troell [1931] was tried [Chakraborty and Sen, 1935] but it was considered unsuitable for inclusion in routine practice. Eventually alkaline permanganate was found to be a satisfactory agent and a new method for the mechanical analysis of all classes of lateritic soils has been developed using this agent as reported below.

Use of alkaline permanganate in oxidation of organic compound is well known [Bigelow, 1919, 1922]. Liquid fatty acids are also oxidized by alkaline permanganate and hydroxylated acids are obtained [Lewkowitsch and Warburton,

1921]. Fallot [1924] made use of potassium permanganate as an oxidizing agent for estimation of humus in soil after extraction of the same with caustic potash. So at the first instance 4 soils of lateritic type containing organic matter from 2·4 to 10 per cent were heated with a solution of potassium permanganate in presence of caustic soda and it was found that in duplicate experiments with a particular soil almost equal quantities of alkaline permanganate were decolorised. The reaction involved in the process of oxidation may be represented thus:—

$$2\,KMnO_4 + KOH + H_2O = 2MnO_2 + 3\,KOH + 3\,O$$

The oxygen liberated oxidises the organic matter. The hydrated manganese dioxide produced as a result of reaction and incorporated with the soil is removed by adding gradually a concentrated solution of sodium bisulphite in the soil acidified with hydrochloric acid. The soil is filtered, washed with sodium acetate solution until free from manganese and sulphate, and finally alkalised to pH 10·5 with normal sodium hydroxide and shaken.

Soil samples

Samples of soils used in this investigation represent the typical Indian laterites. Silica sesquioxide ratios of these samples vary from 1 tc 2 and all of them contain very little calcium carbonate and exchangeable bases.

Solutions used are:—

(1) Normal sodium hydroxide.

(2) Normal potassium permanganate prepared by dissolving 31·6 grms. of permanganate in water and making up to a litre; 50 c. c. of this solution is capable of oxidising ·09 grm. of organic carbon in an alkaline medium.

(3) Normal hydrochloric acid.

(4) 25 per cent sodium bisulphite solution, 5 c. c. of the freshly prepared solution dissolves manganese dioxide produced from 50 c.c. of the above permanganate solution. This solution should not be preserved.

(5) Acidified sodium acetate solution prepared by dissolving 100 grms. of sodium acetate (crystalline) in a litre of water and just acidified before use with normal hydrochloric acid adding a few drops of methyl orange as internal indicator. Calcium sulphate is about 4 times more soluble in this reagent than in water. Hydrated manganese dioxide is also slightly soluble in it.

Experimental

(*a*) *Oxidation of organic matter*

Twenty grms. of air-dry soil are taken in a 600-c. c. Pyrex beaker to which 50 c. c. of permanganate solution and 5 c. c. normal sodium hydroxide are added

successively. The beaker is then placed over a sand-bath and the mixture stirred frequently. As soon as the permanganate colour disappears a fresh addition of 50 c. c. of the permanganate solution is made. This is repeated until the colour lasts for about half an hour after a fresh addition. In soils containing large quantities of organic matter 100 c. c. permanganate may be decolorised within 15 minutes. After the permanent colour has been obtained, 25 c. c. permanganate and 5 c. c. normal sodium hydroxide are added and the reaction completed by heating for half an hour more. Oxidation of humified organic matter is taken as complete when 25 c. c. of permanganate is not decolorised after heating for 30 to 40 minutes. It has been observed that soils containing more than 10 per cent organic matter require less than two hours and a half for this operation while in the case of the majority of the soils reported here oxidation was found to be finished within an hour and a half.

(*b*) *Removal of calcium carbonate*

Although Indian lateritic soils do not contain sufficient calcium carbonate and exchangeable calcium to interfere with direct dispersion with sodium hydroxide, it is advisable to include a process for removing calcium carbonate so that the present method may be applicable to all types of soil. After oxidation of organic matter is complete, the beaker is taken off the sand-bath and allowed to cool. Enough hydrochloric acid is then run in from a burette until evolution of carbon dioxide ceases and the mixture is rendered slightly acid to blue litmus paper. A further addition of 50 c. c. normal hydrochloric acid is made and water is added if necessary, to make the mixture about $N/5$ acid. The contents of the beaker are frequently stirred with a glass rod for about an hour and allowed to stand until the soil settles. The supernatant liquid is passed through a filter and the residue is washed with about 50 c. c. water by decantation. Soils containing unusually large quantities of calcium carbonate should be given a further washing with 50 c.c. of water.

(*c*) *Separation from hydrated manganese dioxide*

Ten c. c. of normal hydrochloric acid for each 50 c. c. of permanganate used for oxidation and 20 c. c. in excess are run into the beaker containing the washed soil from (*b*) and sufficient water is added to adjust the strength of the acid to about $N/2{\cdot}5$ 3 to 4 c. c. lots of 25 per cent sodium bisulphite solution are then added but the contents of the beaker are shaken by hand after each addition. This process is continued until all manganese dioxide is dissolved when the soil looks free from any dark colour. The material in the beaker is allowed to settle and the supernatant liquid is passed through the same filter used in (*b*). Any black particle of manganese dioxide that might have adhered to the filter in course of filtration in (*b*) will be dissolved when it comes in contact with this decanted liquid. Otherwise a dilute solution of bisulphite is to be passed through the filter. To ensure complete removal of manganese dioxide 5 c. c. of normal hydrochloric acid solution and 1 to 2 c. c. of bisulphite solution may be added to the residue in the beaker and shaken for

a few minutes. Treatment with bisulphite in presence of acid should not be carried for a longer period than necessary for removal of manganese dioxide. This operation (*c*) does not require more than 30 to 40 minutes even in soils containing more than 10 per cent organic matter.

(d) Washing free from manganese and sulphate

The soil is transferred to the filter and washed with about 10 c. c. water and then with sodium acetate solution allowing the filtrate to drain off completely before a fresh addition of the acetate solution is made. Washing with this reagent is continued until the filtrate is free from sulphate and manganese. Manganese may be tested by boiling a few drops of the filtrate with a little bromine water and then again boiling with a few drops of ammonium hydroxide when a black precipitate indicates presence of manganese. The presence of sulphate may be detected by the usual barium chloride test in the hot. For most soils washing with 200 to 300 c. c. of acetate has proved quite sufficient. In exceptional cases 400 to 500 c. c. may be required. Finally the soil is washed twice with about 25 c. c. water through a fine jet so as to remove the adhering sodium acetate from the beaker and the filter paper as far as possible.

It was found that filtration and washing in Buchner funnels under pressure is much more rapid and efficient than those carried out in ordinary funnels. For fixing up the filter paper with the Buchner funnel we have used paraffin in place of Durofix cement (cellulose acetate) and found it to be quite satisfactory.

(e) Dispersion and sampling

After the soil has been washed and drained, it is transferred into a 500-c. c. bottle and 8 c. c. of normal sodium hydroxide is added for every 20 grms. of soil used. The bottle is shaken in an end-over-end-shaker at the rate of about 40 revolutions per minute for six hours. The contents of the bottle are made up to 2 litres and subjected to pipette sampling for determination of clay and silt. Coarse sand and fine sand are estimated by sieving and sedimentation respectively. The soil need not be rubbed while passing through 0·2 mm. (No. 70 I. M. M.) sieve.

(f) Determination of loss on solution

An aliquot part generally 1/10 to 1/5 of the filtrate from *b* to *d* (rendered free from permanganate colour if necessary with a few drops of bisulphite) is boiled with a little concentrated nitric acid to oxidize any ferrous iron and sodium bisulphite present in the filtrate. A modified method of determination of sesquioxide in presence of a large quantity of manganese dioxide is followed. The essential feature of this method lies only in using a larger quantity of ammonium chloride and a limited quantity of ammonium hydroxide for precipitation of iron and aluminium. This method has been developed by Lundell and Knowles [1923] and described by Treadwell and Hall [1930] thus:—

"To the acid solution containing 5 grms. of ammonium chloride and not more than 0·2 grm. of iron and aluminium cations in 20 c. c. of solution, add a

few drops of a 0·2 per cent solution of methyl red in alcohol and heat just to boiling. Carefully add normal ammonium hydroxide solution drop by drop until the colour of the solution changes to a distinct yellow. Boil for 2 minutes and filter promptly. Wash with hot 2 per cent ammonium nitrate solution until free from chloride.....As good a separation of iron and aluminium from manganese is accomplished in this way as by a single basic acetate precipitation. It is advisable to dissolve the precipitate and repeat the precipitation when the precipitate on ignition is likely to weigh more than 0·1 grm. or when a 9 cm. filter is more than half full or the precipitate".

Experimental results and discussions

In view of the fact that International Soda method is so far considered as the most satisfactory and rapid method of mechanical analysis applicable to a large variety of soils it was decided to carry out comparison of the clay and clay + silt figures obtained by this method and the method under investigation using 18 typical Indian lateritic soils. It has been pointed out by Robinson [1933] and rightly so that in the case of gypseous soils the International Soda method also needs modification. As there is no gypsum present in the soils under examination, the International Soda method is expected to yield satisfactory results.

TABLE I

Percentage of clay and clay+silt in air-dry soil

Soil No.	Lab. No.	Locality	Loss on ignition	International Soda method H_2O_2-HCl-NaOH (One per cent suspension of 20 grms. soil shaken for 24 hrs. with 8 c. c. *N* NaOH)			Alkaline permanganate method (One per cent suspension of 20 grms. soil shaken for 6 hrs. with 8 c. c. *N* NaOH)		
				Clay	Clay + silt	Loss on solution	Clay	Clay + silt	Loss on solution
1	34	Madras .	16·5	44·4	57·1	0·4	44·2	55·4	1·3
2A	58A	Bombay .	17·5	39·4	53·6	0·43	37·7	53·8	0·97
3	64	Burma .	10·8	18·4	23·3	1·6	19·4	24·7	1·2
4	74	Orissa .	5·8	15·8	35·0	0·92	16·9	36·5	1·3
5	24	C. P. .	9·7	11·8	24·1	0·14	12·1	25·4	0·4
6A	30A	Madras .	25·2*	48·4	59·0	2·2	49·7	62·8	1·4
7	80	Assam .	11·9	29·2	46·8	0·6	28·4	46·6	0·6
10	11	Bihar .	5·5	22·8	36·8	0·3	23·0	37·7	0·5
14	26	Madras .	3·8	11·7	14·7	0·2	11·7	14·8	0·3
15	20	Burma .	4·0	12·1	18·5	0·35	12·2	18·7	0·47
16	38	Bengal .	4·7	18·2	28·2	0·8	18·3	28·2	0·45
17	44	Bengal .	2·8	9·7	24·3	0·24	9·8	24·4	0·4
19	88	Assam .	22·7	31·3	61·9	3·7	33·7	65·7	1·7
20	56	Bombay .	18·3	45·2	59·6	1·0	45·4	61·5	0·94
21	60	Burma .	13·5	48·2	61·8	0·7	46·8	60·1	1·2
22	66	Bihar .	3·5	14·0	22·7	0·5	14·3	22·9	0·6
23	76	Orissa .	11·3	41·2	62·8	0·7	40·5	63·7	0·96
24	86	Assam .	4·3	12·5	22·2	0·46	12·7	22·2	0·6

*Containing 10 per cent organic matter

A perusal of Table I clearly indicates that the agreement between the two sets of figures by the two methods are on the whole very good. The alkaline permanganate method is therefore as efficient as the International Soda method in effecting maximum dispersion of soil. Further, the percentage loss on solution in the permanganate method did not exceed that in the International Soda method.

Time of shaking required for complete dispersion

Six soils rich in organic matter were chosen and their clay figures were estimated by the alkaline permanganate method shaking the final suspension to varying lengths of time from 3 to 24 hours. The results are given in Table II below from which it will appear that it is not necessary to shake for more than six hours with this method.

Table II

Percentage of clay in air-dry soils

Soil No.	Hours of shaking			
	3	6	12	24
2A . . .	37·9	37·7	37·9	38·3
3	19·3	19·4	19·4	19·2
5	12·6	12·1	12·6	13·4
6A . . .	47·1	49·7	49·4	49·9
19	33·9	33·7	33·5	33·7
20	45·1	45·4	45·6	46·3

Soils to which large quantities of calcium carbonate and calcium sulphate have been added

As no soil rich in either lime or gypsum was available in this laboratory it was thought to test the applicability of alkaline permanganate method in samples of soils mixed with large quantities of calcium carbonate and calcium sulphate. It is admitted that the behaviour of these soils may not be the same as that of soils rich in lime or gypsum in the natural state, but it is thought that some idea as to the interference to dispersion, if any, due to lime and gypsum may be obtained from experiment with these mixed soils. 20-grm. lots of four soils were taken. One lot was left untreated while 4 grms. of calcium carbonate were added to each sample of the second lot and 4 grms. of calcium carbonate + 2 grms. of calcium sulphate to each sample of the third lot.

The results of analysis of these soils by the alkaline permanganate method are given below.

TABLE III

Soil No.	Loss on ignition	Per cent clay (Untreated soil)	Per cent clay soil + calcium carbonate	Per cent clay soil + calcium carbonate + calcium sulphate
2A . . .	17·5	37·7	37·7	36·7
3	10·8	19·4	19·0	19·1
6A . . .	23·2	49·7	48·4	48·6
19	22·7	33·7	32·9	34·0

It is clear from the table that maximum dispersion is obtainable by the alkaline permanganate method whether the soil contains calcium carbonate and calcium sulphate or not. This is probably due to the fact that greater part of the gypsum present in the soil is transformed into calcium carbonate as a result of reaction between calcium sulphate and alkali carbonate produced during oxidation of organic matter with alkali permanganate. The reaction may be represented thus:—

$$Na_2CO_3 + CaSO_4 = Na_2SO_4 + CaCO_3$$

The calcium carbonate formed along with that originally present in the soil is removed by the subsequent hydrochloric acid treatment followed by washing. On the other hand, washing with sodium acetate solution included in the method also helps to remove gypsum to a great extent, for the solubility of calcium sulphate in the acetate solution is found to be 4 times greater than in water. It must be pointed out however, that for soils containing gypsum sodium hydroxide as demanded by the above equation (15 c. c. normal caustic soda for each one grm. calcium sulphate) should be added gradually in addition to the quantity of alkali used with permanganate to remove organic matter. Coarse gypsum present in gypseous soils should be removed by passing the soil through a 70-mesh sieve after oxidation with alkali permangnate and before addition of hydrochloric acid [Robinson, 1933].

Finally it may be mentioned that although the alkaline permanganate method has been shown here to be quite suitable for the mechanical analysis of Indian lateritic soils, it is expected that the method will be found equally suitable for all other types of soil.

SHORT DESCRIPTION OF THE ALKALINE PERMANGANATE METHOD FOR THE MECHANICAL ANALYSIS OF LATERITIC SOILS

Twenty grms. of soil are taken in a 600-c. c. beaker to which 50 c. c. of 3·16 per cent potassium permanganate and 5 c. c. of normal caustic soda are

added. The beaker is then placed on a sand-bath and the mixture stirred frequently. As soon as the colour disappears a fresh 50 c. c. of permanganate solution is added. This is repeated until the colour lasts for half an hour when a further addition of 25 c. c. permanganate and 5 c. c. normal caustic soda is made and the whole thing heated again for 30 to 40 minutes to bring oxidation to a finish.

The beaker is then allowed to cool. Sufficient normal hydrochloric acid is run until evolution of carbon dioxide ceases and the mixture becomes slightly acid. Further 50 c. c. of normal hydrochloric acid is added and the strength of the acid in the beaker is adjusted to $N/5$ with water. The contents of the beaker are frequently stirred for one hour and allowed to settle. The supernatant liquid is filtered off and the residue in the beaker is washed with about 50 c. c. water by decantation to remove greater part of calcium salts.

Ten c. c. of normal hydrochloric acid for each 50 c. c. of permanganate used and 20 c. c. in excess are poured into the beaker and an equal quantity of water is added. Twenty-five per cent solution of sodium bisulphite added to the material in the beaker in doses of 3 to 4 c. c. stirring frequently after each addition until all manganese dioxide is dissolved.

The mixture is allowed to settle and the supernatant liquid is poured on the filter used previously. The residue in the beaker is transferred to the filter and washed with a little water and then with 10 per cent sodium acetate solution, just acidified with hydrochloric acid, until the filtrate is free from manganese and sulphate. The soil is drained and finally washed twice using 25 c. c. water each time.

The residue in the funnel are transferred to a 500-c. c. bottle by means of a jet of water and shaken for 6 hours in a rotary shaker moving at a speed of about 40 revolutions per minute with 8 c. c. of normal caustic soda.

The soil suspension is made up to 2 litres and subjected to pipette sampling.

Summary

(1) A new method for the mechanical analysis of lateritic soils has been developed using alkaline permanganate to remove organic matter.

(2) The results for clay and clay plus silt obtained by this method in the case of 18 lateritic soils from all over India were comparable to those obtained by International Soda method.

(3) The loss on solution in this method is not greater than that in the International Soda method.

(4) Shaking for six hours in a rotary shaker is sufficient for complete dispersion of soil by this method.

(5) This method without modification was able to disperse completely four lateritic soils in which large quantities of calcium carbonate and sulphate have been added.

(6) The method is described fully.

(7) Advantages of the method are:—

(i) Alkaline permanganate is equally suitable in temperate and tropical countries where hydrogen peroxide is less stable.

(ii) It can be used in manganiferous soils where hydrogen peroxide is required in large quantities or is useless.

(iii) Time required for oxidation does not exceed three hours even in soils containing large quantities of organic matter (*e.g.*, 10 per cent). On the other hand hydrogen peroxide and sodium hypobromite require much longer time for the purpose.

(iv) Obnoxious and injurious chemicals like bromine, as in the method using sodium hypobromite, are avoided.

(v) This reagent is much cheaper than either hydrogen peroxide or sodium hypobromite.

(8) Although the alkaline permanganate method has been shown here to be quite suitable for the mechanical analysis of Indian lateritic soil, it is expected that the method will prove equally suitable for all other types of soil.

ACKNOWLEDGMENTS

The author wishes to express his thanks to Dr. A. T. Sen, under whom this work has been carried out, for valuable suggestions and criticisms; to Prof. J. C. Ghosh for taking keen and continued interest in the progress of the work; and to the Imperial Council of Agricultural Research for financial help towards the purchase of necessary apparatus and maintenance of his salary.

REFERENCES

Bigelow, L. A. (1919). *J. Amer. Chem. Soc.* **41**, 1559.

——————— (1922). *J. Amer. Chem. Soc.* **44**, 2010.

Chakraborty, J. N. and Sen, A. T. (1932). *Ind. J. Agric. Sci.* **2**, 516.

——————————————— (1935). *Ind. J. Agric. Sci.* **5**, 39.

Fallot, B. (1924). *Chim. et. Ind.* **11**, 873.

Lewkowitsch, J. and Warburton, G. H. (1921). "Chemical Technology and Analysis of Oils, Fats and Waxes" 6th edit. Vol. 1, pp. 228 and 575. Macmillan & Co., Ltd., London.

Lundell, G. E. F. and Knowles, H. B. (1923). *J. Amer. Chem. Soc.* **45**, 676.

Read, J. W. and Riddgell, R. H. (1922). *Soil. Sci.* **13**, 1.

Robinson, G. W. (1933). *Imp. Bur. Soil. Sci., Tech. Com. No.* **26**.

Treadwell, F. P. and Hall, W. T. (1930). "Analytical Chemistry," 7th edit. Vol. II, p. 156. Jhon Wiley and Sons, Inc., N. Y.

Troell, E. (1931). *J. Agric. Sci.* **21**, 476.

STINKING SMUT (BUNT) OF WHEAT WITH SPECIAL REFERENCE TO *TILLETIA INDICA* MITRA

BY

M. MITRA, M.Sc., PH.D., D.I.C.,

Assistant Mycologist, Imperial Institute of Agricultural Research, Pusa, Bihar

(Received for publication on 23rd July 1934

(With Plates I–VII and two text-figures)

CONTENTS

I. INTRODUCTION

Stinking smut or bunt of wheat was until recently believed to be caused by two closely related species of Tilletia *viz.*, *T. caries* (DC.) Tul. [*T. tritici* (Bjerk.) Wint.] and *T. foetens* (Berk. and Curt.) Trel. (*T. laevis* Kuehn.), but diseased seeds of certain wheat varieties collected at Karnal (Punjab) in May 1930 showed the presence of an undescribed species of this genus differing from *T. caries* and *T. foetens* in several respects. It was named by the author as *T. indica* and a brief description has already been published [Mitra, 1931,1].

In India, three species of Tilletia are known to occur and their distribution is as follows:—

T. caries.—Gilgit (Kashmir), Sopor (Kashmir), Simla Hills and Kangra (Himalayan range).

T. foetens.—Gilgit (Kashmir), Sopor (Kashmir), Jubbal (Simla Hills) and Baluchistan (Himalayan and Sulaiman Mountain ranges).

T. indica.—Karnal, Lahore, Amballa, Jaranwalla, Lyallpur and Ropar (Punjab) and Peshawar (North Western Frontier Province).

It will be noticed from the distribution of the three species that so far as it is known at present, bunt caused by *T. caries* and *T. foetens* is confined to the cooler regions of the Himalayas and Baluchistan, while *T. indica* occurs only in the plains of the Punjab and the North Western Frontier Province.

Howard and Howard [1909] recorded the occurrence of bunt on wheat at Lyallpur. *T. indica* has been found at that locality and the neighbouring districts in the plains of the Punjab during the last four years. It is likely that the bunt observed by Howard and Howard as early as 1908 was caused by this species.

The author has not been able to find any specimen of *T. indica* in the herbarium collection of bunts of wheat preserved at the Imperial Institute of Agricultural Research, Pusa. This shows that this bunt was never even collected before.

It has been found that low temperatures are most favourable for the infection of susceptible wheats by *T. foetens* and *T. caries*. At temperatures much above 10°C., the percentage of infection falls off rather rapidly and few seedlings grown at temperatures higher than 20°C. are infected. Munerati [1922] obtained 90-100 per cent of bunt with *T. caries* and *T. foetens* in wheat germinated at 7-8°C. while the same varieties gave 1·4-12 per cent infection when germinated at 18-20°C. The same author [Munerati, 1923] found that seeds germinated at 10-12°C. gave 13·2-39·3 per cent infection while the same two varieties produced 0-1·4 per cent bunt at 22-25°C. Similar results were obtained by Volkart [1906], Heuser [1922] and Hungerford [1922]. Faris [1924] states that infection does not take place at 20°C., 25°C. and 30°C. with *T. caries* and very rarely at 20°C. and 25°C. with *T. foetens*. He obtained successful infection with *T. caries* at temperatures between 5 and 15°C. (mostly at 5-10°C.) and with *T. foetens* between 5 and 25°C. (mostly at 10-15°C.) and very rarely at 25°C. Tisdale, Leighty and Boerner [1927] in their studies of the distribution of *T. caries* and *T. foetens* have also shown that temperatures ranging from 5 to 15°C. are very favourable for infection.

The conclusion drawn by these authors is that *T. caries* is more generally distributed throughout the cooler regions and that *T. foetens* can tolerate slightly higher temperatures. Thus it is clear that for infection with *T. caries* and *T. foetens* the temperature should not be above 15°C. and that temperature plays an important part in the distribution of these bunts. *T. caries* and *T. foetens* require a much cooler climate to thrive in than *T. indica*. That seems to be the reason why *T. caries* and *T. foetens* are not found on the plains of North-West India where this low temperature generally does not occur at the time of sowing or harvesting wheat and this accounts for their absence from localities where the average temperature is above 20°C. at the time of sowing and above 30°C. at the time of harvesting. The occurrence of *T. indica* on the plains indicates that this species can stand warm weather. At Karnal the average maximum temperature at the time of sowing of wheat was 34·5°C. during 1932-33 and 29·5°C. during 1933-34. Though the minimum goes down to 14°C., the number of hours per day having more than 20°C. temperature is far more than in the hills.

Further, it may be mentioned that the weather following the harvest in the hills where *T. caries* and *T. foetens* occur is cool whereas on the plains of North-West India where *T. indica* alone occurs, the wheat season is followed by a hot summer.

II. Losses due to stinking smut (bunt)

There is very little information available regarding the damage done to wheat by bunt in India. It appears however that *T. caries* and *T. foetens* do a good deal of damage to wheat in Kashmir, Baluchistan and in the Simla Hills. In 1933 it was reported from Jubbal (Simla Hills) that the damage due to *T. foetens* was from 30-40 per cent. In 1923 bunt was reported to be very bad at Peshawar and for the first time did considerable damage to both the bearded and beardless varieties. As no specimens were sent to Pusa, it was not possible to determine the species of Tilletia responsible for the loss. During 1932, it was again reported to have done considerable damage to the wheat crop, especially the beardless varieties, at Peshawar, and the loss was estimated to be up to 33 per cent in several cases. Specimens of bunted grain showed the presence of *T. indica* and most probably the species responsible for the loss during 1923 at Peshawar was the same, as the other two species have so far not been recorded from that locality.

T. indica was first observed at Karnal on certain hybrids (cross between Federation and Pusa 4 and Pusa 52), *viz.*, Pusa 4 × Fed. (1—1), Pusa 4 × Fed. (7—1), Pusa 4 × Fed. (97—1), Pusa 4 × Fed. (64—1). Pusa 4 × Fed. (3—1) = P. 165, Pusa 52 × Fed. (20—1) = P. 120, Pusa 52 × Fed. (38—1), Pusa 52 × Fed. (64—1). During 1931 it was further observed on Pusa 4, 52, 114, 115, 116, 117, Federation and Punjab 8A. During 1933, it appeared in a virulent form at Karnal on several of the wheat varieties Pusa 4, 12, 52, 114, 165 were slightly attacked, Pusa 80—5, 111, 120 had infection up to 2 per cent and Pusa 112 and 113 showed nearly 10 per cent Seed of Pusa 72—1—1 was threshed at the time of examination and showed more than 20 per cent diseased grain.

The estimate of the percentage of infection is based on the number of ears having bunted grain. The loss in yield thus is much less, as only a very few spikelets in an ear are attacked.

The presence of *T. indica* on Punjab 8A indicated the probability of its being present in other parts of the Punjab. Consequently an opportunity was taken to examine wheat in the Lahore grain market where it is stored in large quantities from different parts of the province and bunted grain was found to be fairly common. The disease is therefore fairly widespread throughout the plains of that province.

III. Symptoms produced by *T. indica*

The previous description of this fungus [Mitra, 1931] was based on a small collection of bunted grain picked out after threshing. The disease being rather severe during 1933 at Karnal, an opportunity was taken to make more detailed observations.

In a stool all the ears are not infected and in an ear all the grains are not bunted. Thus the same plant produces sound and bunted ears and the same ear, sound and bunted grain. A completely infected ear has never been observed, the sori being only produced in a few individual spikelets; generally 1—5 spikelets show bunted grain in an ear while the rest produce healthy grain. It is difficult frequently to detect diseased grain in an ear without breaking the spikelets, as the grain is hidden by the enclosing unaffected glumes. This is especially the case in unripe ears. When the grain ripens the diseased spikelets are more open, the outer glumes spread out giving more space to the inner glume and the palae to expand with the result that the bunted grain becomes visible between the glumes. In early stages, a careful examination of the embryo portion of the seed helps to detect the disease where a black spot on the grain is noticeable at the tip of the spikelet. Often the grain is very slightly infected at the tip and the presence of the fungus can be noticed only with the help of a lens. In badly infected spikelets the glumes spread apart and later on fall off, thus exposing the bunted grain which also falls to the ground with a slight disturbance and infects the soil. Plate I illustrates the presence of bunted grain in ears.

No smell was detected in the small samples previously collected and recorded [Mitra, 1931]. Because of the abundance of material collected during 1933 it was possible to find out that this bunt like the other two has also a distinct stinking smell resembling that of rotten fish and the smell is more pronounced when the bunted grain is crushed.

The bunt affects the kernels only partially, which are not swollen. The embryo tissue except in very severe cases is not destroyed though in a large number of cases only the embryo portion is infected. Generally the infection spreads to the tissue along the groove but the endosperm material lying along the smooth side of the grain is uninfected. Bunted grain has been found to germinate freely, the percentage of germination being as high as eighty-nine in several cases. Further, they germinate more quickly than the healthy grain. *T. indica* is more highly differential than *T. caries* and *T. foetens* which leave only the glumes and the epidermal tissues of the kernels uninfected. The various types of infection of the grain from mild to severe are illustrated in Plate II.

Sori are formed under the pericarp and testa above the aleurone layer of the endosperm and are brown to dark brown in colour (Plates III, IV and V figs. 1 and 2). The attack starts at the embryo end and runs along the groove leaving the endosperm intact covered by the whole or partly ruptured pericarp. Gradually the endosperm region is replaced by the fungus. At first the spores in sori are enclosed by the pericarp and cannot be seen from outside and only microscopic examination (transverse and longitudinal sections) reveals its presence (Plates IV and V, fig. 2). Later on the pericarp ruptures and the spore mass is exposed. (Plates III and V, fig. 1). A collection of bunted grain shows all stages of infection from very slight to severe.

IV. Morphology of *T. indica*

The spores bearing hyphae in a sorus give off pear-shaped bud clusters (Plate III and Plate V, fig. 1). These outgrowths increase in diameter and become rounded so that they are ultimately attached to the hyphae by a thin stalk. The membrane is gelatinous in young spores and disappears on maturity. The stalk or the thread of detachment sometimes remains attached to the spore. In transverse and longitudinal sections of diseased grains the fungal filaments and numerous spores are interspersed between the outer skin and the aleurone layer on the under-surface. The embryo remains quite intact though partially surrounded by numerous spores.

Spores when mature are dark brown, black in mass, spherical, subspherical or oval, 22—44 × 25—55 μ in diameter, average 32·5—40 μ. The proliferations of the thick epispore are reticulate, the ridges or scales are somewhat roundish, or bluntly projecting showing at the circumference of the spore a band 2—6 μ in width enveloped by a clear membrane probably gelatinous. Mixed with spores are numerous yellowish or sub-hyaline sterile cells, rounded, angular or pear-shaped, smaller in size than the spore, relatively thin-walled, and occasionally small brown cells occur which are smooth-walled and much smaller than spores. These in part represent undeveloped spores. The spores often have a papilla or thread of detachment. The spore mass dries up into pustules which are capable of being blown about by wind. Plate V, fig. 3, Plate VI, fig. 1 and Plate VII, figs. *a*, *b* and *c* illustrate the shape and details of spores.

The spores of *T. indica* differ considerably from *T. foetens* which has smooth-walled spores, much smaller in diameter and lighter in colour. Spores of *T. caries* resemble those of *T. indica* in the structure of their walls but they are much smaller in size and lighter in colour than those of the latter.

Spores of *T. caries* are dusty olive brown or rust coloured in mass while those of *T. indica* are black. In *T. caries* sterile cells are few while in *T. indica* they are numerous. In *T. caries*, the whole tissue is infected and except the outer coat everything is destroyed and partly infected grain is rare [McAlpine, 1910 and Heald, 1916] while in *T. indica* partly infected grain is very common and the whole tissue is seldom destroyed and completely infected grain is very uncommon.

V. Variation in spore size of *T. caries* and *T. indica*

Brentzel [1933] recently presented evidence to show that there existed a wide variation in the size and shape of spores and in the degree of spore-wall reticulations of *T. caries*. In the photograph which he published there were also present some unusually dark spores which appeared to resemble in appearance the spores of *T. indica*. It was thought advisable therefore to compare the spores of *T. indica* with those illustrated by Brentzel. Dr. Brentzel very kindly supplied the material from two collections, one from Ceres wheat (originally from Emmer) and the other from Kota wheat (originally

from Mindum, durum). For the sake of convenience the strain on Ceres is hereafter referred to as the Emmer strain and the other on Kota as the Durum strain.

For biometrical analysis of the spore sizes and their comparisons two hundred spores from each collection were measured. In addition to the two strains of *T. caries* supplied by Brentzel, a collection of *T. caries* from Gilgit (Kashmir) and two collections of *T. indica*, one from Karnal and the other from Peshawar, were included in the measurements. The frequency distributions for length and width (Figs. 1 and 2) and the biometrical constants are recorded in Tables I and II.

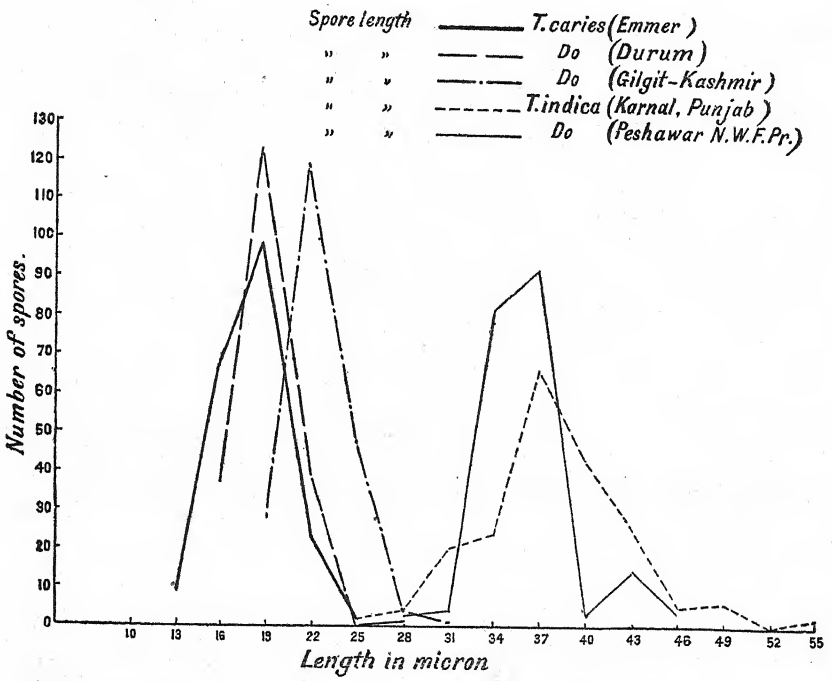


Fig. 1.—Comparison of spore length of three forms of *T. caries* and two forms of *T. indica*.

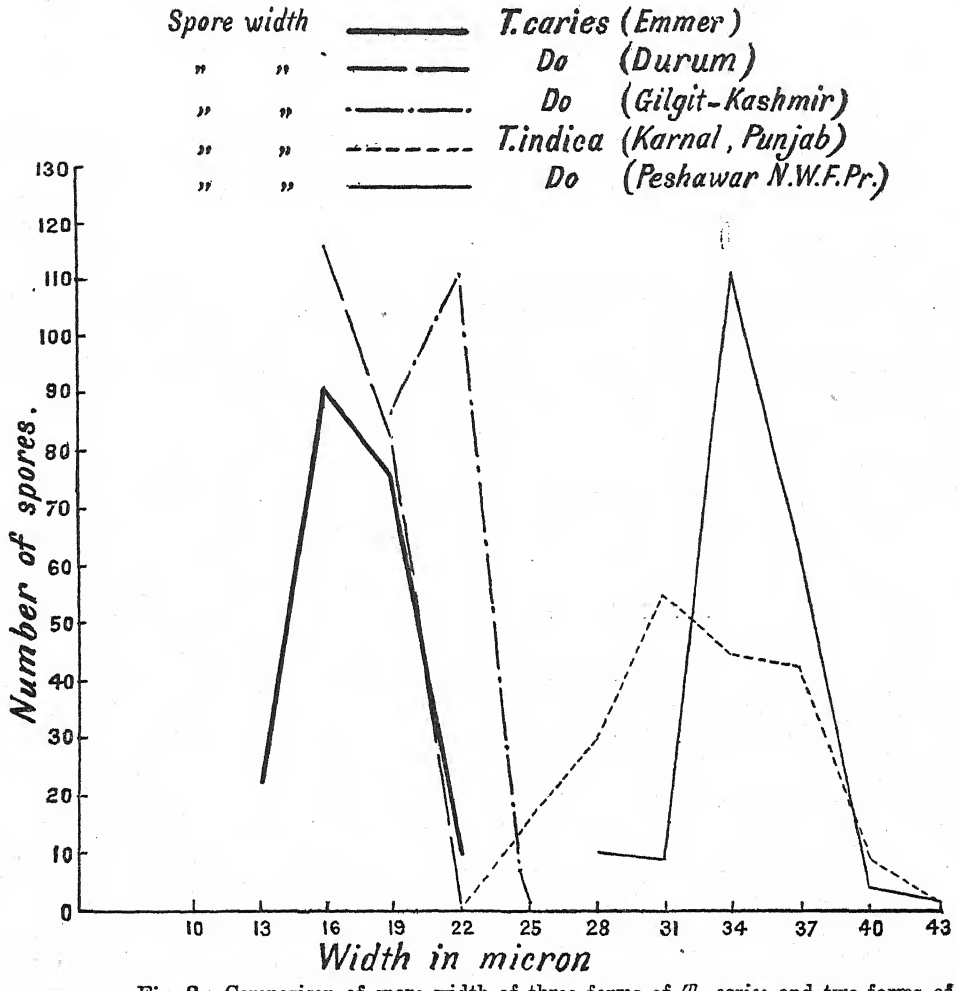


Fig. 2.—Comparison of spore width of three forms of *T. caries* and two forms of *T. indica*.

TABLE I

Variations and constants of spore length and width of three forms of T. caries

Bunt form	Spore length and width classes (in microns)							Range	Mode	Constants		
	13	16	19	22	25	28	31			Mean	S. D.	C. V.
Emmer L	9	67	99	23	2	...	...	12—25	19	18·1±·10	2·12±·07	11·7 ±·39
„ W	22	91	77	10	...	...	...	12—23	16	17·1±·09	2·05±·06	12·02±·40
Durum L	...	37	123	39	...	1	...	15·4—27·5	19	19·1±·08	1·75±·05	9·17±·30
„ W	...	116	83	1	...	...	...	15·4—22	16	17·3±·06	1·43±·04	8·28±·27
Gilgit L	...	...	28	119	48	4	1	18—30	22	22·6±·09	1·89±·06	8·42±·28
„ W	...	...	88	111	1	...	...	18—24	22	20·7±·05	1·25±·04	6·04±2·03

TABLE II

Variations and constants of spore length and width of two forms of T. indica.

Bunt form	Spore length and width classes (in microns)												Range	Mode	Constants		
	22	25	28	31	34	37	40	43	46	49	52	55			Mean	S. D.	C. V.
Karnal L	...	2	4	20	24	66	43	28	5	6	...	2	22—55	37	37·9±·22	4·75±·16	12·51±·42
„ W	1	16	30	55	45	43	9	1	...	...	...	...	22—42	31	32·5±·19	3·98±·13	12·28±·41
Peshawar L	...	...	3	4	81	91	3	14	4	...	...	...	27·5—46·2	37	36·2±·13	2·90±·09	8·02±·27
„ W	...	...	10	9	112	64	4	1	...	...	...	...	27·5—44	34	34·7±·10	2·24±·07	6·45±2·17

(L = Length, W = width)

From the data recorded in Tables I and II, it will be noted that the range of spore length and width of the Emmer strain is 12–25 μ ×12–23 μ with a mean of 18·3 μ ×17·1 μ, that of Durum strain is 15–28 μ ×15–22 μ with a mean of 19·1 μ ×17·3 μ and that of the Gilgit strain is 18–30 μ ×18–24 μ with a mean of 22·5 μ ×20·7 μ. The range in the case of the Karnal collection of *T. indica* is 25–55 μ ×22–42 μ with a mean of 37·9 μ × 32·5 μ and that of the Peshawar collection 27—46 μ × 27—44 μ with a mean of 36·2 μ ×347μ.

The above figures indicate therefore that apart from the differences already stated in the section on morphology, the spore sizes of the two species are absolutely different, that is, spores of *T. indica* are almost double the size of those of *T. caries*.

Furthermore, the data recorded in Tables I and II indicate that even within the species themselves, there is evidence of the occurrence of different physiologic forms. The mean length and width of spores from three collections of *T. caries* and two of *T. indica* showed considerable differences and in order to know whether the respective mean differences were statistically significant or not, Pearson's method of comparing the frequencies was applied. The results are recorded in Table III.

TABLE III

Probability for or against the different collections of T. caries *and* T. indica *being the same physiologic forms*

Fungus	Collections compared	Length or width	χ^2	Odds	Remarks
T. caries .	Emmer and Gilgit .	Length .	227·88	Odds against very high	Not same.
	,, ,, .	Width .	199·92	,,	,,
	Emmer and Durum.	Length .	27·32	,,	,,
	,, ,, .	Width .	32·60	,,	,,
	Durum and Gilgit .	Length .	188·04	,,	,,
	,, ,, .	Width .	229·12	,,	,,
T. indica .	Karnal and Peshawar	Length .	95·27	,,	,,
	,, ,, .	Width .	85·64	,,	,,

Odds in each and every case are very highly against their being the same physiologic forms. The Emmer, Durum and Gilgit strains of *T. caries* could only be considered therefore as physiologic forms. According to Stakman [1929] there are four general methods for recognising physiologic forms, cultural characters, physico-chemical reactions, pathogenicity on certain selected plants and sometimes morphology; but the spore size which is a characteristic and definite morphologic feature shows wide variations even within the same species in the above-mentioned cases. So far as the Peshawar and Karnal strains of *T. indica* are concerned, although physiological, physico-chemical and parasitic specialization studies have not so far been carried out, yet there are hardly any morphological differences except significant variation in their spore sizes to distinguish them as two different species. The writer however would like to adopt the more conservative view of this question and consider them as merely physiologic forms even though the spore size is so considerably different. Furthermore, the writer would like to emphasize that whereas spore length and width are definite characters of specific value, the differences should not be interpreted too narrowly. The writer has shown [Mitra, 1931,2] that saltants arise from monoconidial cultures of Helminthosporium sp., and that they show considerable variation in spore colour, septation, size, etc. Biometric analysis of spore measurements should therefore be taken as an additional criterion by which physiologic forms may be determined.

VI. Spore germination of *T. indica*

The most favourable conditions for profuse spore germination of *T. indica* have so far not been discovered. Freshly collected spores generally do not germinate and it appears they require a period of rest. Very few spores germinated under various conditions tried, such as, in liquid media like distilled, tap and rain water; one per cent solutions of cane sugar, asparagin, malt extract or soil extract. Even on solid media, such as, plain agar, Brown's synthetic agar, synthetic starch agar and potato juice agar, the germination was extremely poor.

Bunt was kept in ice from 10–45 days and tested weekly but did not show better germination; on the other hand material kept at 35°C. in an incubator for ten days germinated slightly better and more quickly than the frozen material.

Whenever the spores germinated, the germination took place at a temperature ranging from 15—25°C. Those kept at 10°C. or below or at room temperature of 32°C. during summer did not germinate.

When the spore germinates, the epispore ruptures before the promycelium comes out into which the protoplasm of the spore passes. The promycelium varies in length from 10 μ or less to something like 1·5 mm. (1,500 μ) or more. Generally it is unbranched but sometimes branched promycelium has also been noticed. The promycelium has a tendency to go on elongating and as it elongates the basal portion degenerates. The contents of the promycelium are

coarsely granular and the protoplasm moves continuously forward, so that the protoplasm is confined to the upper portion, the portion next to spore becomes vacuolated, and later entirely vacant when septa are laid down. At the tip of the promycelium a crown of slender sporidia is formed in a whorl. The formation of sporidia is often delayed on account of the continuous growth of the promycelium. The number of sporidia varies from 32 to 128 and even more.. (Plate VI, figs. 2 and 3 and Plate VII figs. *d*—*h*). Fusion of two sporidia has never been noticed. Spores germinating in hanging drop cultures or on solid media were kept for more than a month but no fusion of sporidia was noticed and no further growth took place.

VII. Infection experiments carried out at Pusa and Karnal

It has already been mentioned that *T. indica* does not occur nor has so far been recorded from places outside North-West India. The writer has been making careful observations for this bunt for the last four years in Bihar but has not come across a single bunted grain in any grain market of that province. Hundreds of maunds of wheat were carefully examined every year in the Botanical Section and the Farm at Pusa but no trace could be obtained of this bunt. Even seed from Karnal sown at Pusa never showed any sign of infection. It was also observed that partly bunted grain of *T. indica* does germinate and produce seedlings. In order to verify the observations made and to see whether it is possible to produce bunt in Pusa under ordinary climatic conditions by sowing naturally infected seeds at the normal sowing time or by sowing late when the temperature has cooled down, the following experiments were carried out in soil, in pots and in plots.

Experiment I.—Bunted grains of five wheat varieties collected during May and June 1930 were sown in Pusa in sterilized soil in pots in November. There were ten pots for each variety and each pot had six seedlings, that is, altogether fifty pots and 300 seedlings. At maturity during April 1931, all the plants formed normal ear and produced healthy grains. Seeds of the same varieties kept under similar conditions when sown in Karnal produced bunt.

Experiment II.—A parallel experiment was carried out at Pusa in which diseased seeds obtained from Karnal were given an extra dose of artificial infection by mixing the seed with spore powder. The spore powder was obtained at the time of infection by crushing naturally infected grain kept under dry conditions. None of the ears of the 300 wheat plants produced a single bunted grain.

Experiment III.—In order to see if sowing in the cold weather or when the climatic conditions are somewhat like those of Karnal help in producing bunt at Pusa, diseased seeds of Pusa 113 collected during May 1933 at Karnal were sown at different intervals during November and December of the same year at Pusa and the details are given in Table IV.

TABLE IV

Infection experiment in sterilized soil in pots with bunted grain during 1933-34

Date of sowing	No. of pots	No. of plants	Infection
2nd November 1933	20	100	*Nil*
20th „ „	20	100	„
29th „ „	20	100	„
6th December „	20	100	„

The crop was harvested in April 1934 and each and every ear was threshed by hand and every grain was carefully examined but not a single bunted grain was noticed. The seeds of this same wheat type kept under similar conditions when sown in Karnal had three per cent bunted ears.

Experiment IV.—The diseased seeds of four wheat varieties collected at Karnal during May and June 1933 were sown in a plot at Pusa on 5th December 1933, that is, five or six weeks after the normal time of sowing. The idea of sowing late was to secure as far as possible climatic conditions prevailing at Karnal at the sowing time. Each variety was sown in several plots. Pusa 113 seed from the Botanical Section, Pusa, was also sown in a contiguous plot. The number of ears in all the plots were counted and final observations were made at the time of maturity. The details are given in Table V.

TABLE V

Infection experiment with bunted wheat grain during 1933-34 at Pusa under field conditions

Wheat variety	Replications	No. of ears	No. of bunted ears	Remarks
Pusa 113	1	894	*Nil*	Seed from Botanical Section, Pusa & free from bunt
	2	710	,,	
	3	640	,,	
	4	638	,,	
Pusa 113	1	620	,,	Seed from Karnal & heavily infected with bunt
	2	596	,,	
	3	651	,,	
	4	1,189	,,	
Pusa 113	1	423	,,	Selected heavily infected seed from Karnal
	2	618	,,	
Pusa 4	1	331	,,	Ditto.
	2	493	,,	
	3	452	,,	
	4	559	,,	
	5	577	,,	
Pusa 72-1-1 . . .	1	498	,,	Seed from Karnal and heavily infected with bunt
	2	567	,,	
	3	523	,,	
	4	649	,,	
Pusa 165	1	462	,,	Selected heavily infected seed from Karnal
	2	586	,,	
	3	490	,,	
	4	436	,,	
	5	436	,,	

At the time of harvesting at the end of April 1934 each and every head was examined and threshing was done by hand but not a single infected grain was noticed.

Experiment V.—During 1932-33 bunt was up to twenty per cent on Pusa 72-1-1 at Karnal. Part of this seed was brought to Pusa and sown as shown in Table V. The total number of ears obtained in Pusa from four plots in which this variety was sown was 2,237 and none produced bunted grain. The seed from the same lot was also sown in Karnal in twelve plots and the total number of ears counted was 8,535, out of which 455 ears produced bunted grain giving 5·33 per cent of infected ears. The details are given below in Table VI.

TABLE VI

Infected seed of Pusa 72-1-1 sown at Karnal

Replications	No. of ears	No. of ears bunted	Percentage
1	584	28	4·79
2	670	32	4·77
3	695	34	4·89
4	765	36	4·70
5	870	42	4·82
6	670	33	4·92
7	618	36	4·82
8	750	44	5·86
9	895	53	5·92
10	611	35	5·72
11	720	42	5·83
12	686	40	5·83
Total .	8,535	455	5·33

Experiment VI.—An experiment similar to Experiment V was carried out at Karnal using Pusa 113 from the same lot from which it was used in Experiment IV and shown in Table V. The total number of ears produced at Pusa was 2,956 from four plots and none produced bunted grain whereas at Karnal this type was sown in five plots and the total number of ears counted was 4,767, out of which 50 ears produced bunted grain giving 1·1 per cent of infected ears.

Seeds of Pusa 165 which were brought to Pusa from Karnal and sown in five plots had no bunted grain whereas same seed at Karnal produced a fair amount of bunt.

Thus the seeds of Pusa 72-1-1, 113 and 165 which were stocked at Karnal and brought to Pusa at the time of sowing produced bunt at Karnal and not at Pusa. This shows clearly that climatic conditions at Pusa are responsible for the failure of infection.

Experiment VII.—In addition to the above experiments a very large number of diseased seeds were kept in an incubator at 22°C. and when germinated were sown partly in the end of November and partly early in December in pots.

Seedlings when four to six inches in height were taken out and microscopically examined. In several of them mycelium similar to that of a bunt was noticed especially at the nodes. No mycelium was found in grown up plants. The plants left after examination and allowed to form ear produced no bunt.

Experiment VIII.—In order to see whether the bunt is in a dormant state on wheat in Pusa, that is, whether it will produce infection if favourable conditions are given, seeds of Pusa 111, 112 and 113 from the Botanical Section, Pusa, were taken to Karnal and sown side by side with Karnal seed. Each variety was sown in twelve plots and each plot had on an average 650 ears. Minute examination of individual grain revealed total absence of bunt. The crop was hand-threshed and grains of all the thirty-six plots were very carefully examined but no bunt was noticed.

Pusa 113 (Karnal seed) was also sown side by side in twenty-four plots and showed approximately 2·5 per cent of bunted ears. This shows that Pusa seed is free from the disease.

The fact that the disease has never been observed at Pusa or in other parts of Bihar, and the non-occurrence of the disease when Pusa seed is sown at Karnal or when Karnal seed is sown at Pusa support the view that the climatic conditions prevailing in Bihar are unsuitable for the spread of this disease. Further, seeds kept under identical conditions were able to produce bunted grain at Karnal and not at Pusa. Late sowing when the temperature lowers also does not help the bunt to appear at Pusa. By late sowing the crop is harvested late and it appears that high temperature at the time of maturity is unfavourable for the bunt to grow in the tissue of the host plant.

VIII. Experiments on control measures

In order to determine the effect of some fungicides on the incidence of *T. indica* an experiment was carried out at the Botanisal Sub-Station, Karnal, in 1933-34. The fungicides tested were copper carbonate, ceresan, formalin and uspulun (universal). Untreated wheat seed was also sown as control, thus making up five treatments in the experiment. Three varieties of wheat used were Karnal-grown seed of Pusa 111, 112 and 113. There were two methods of infection, *viz.*—

Series A. Naturally infected seed

Series B. Naturally infected seed plus an extra dose of artificial infection by shaking every 100 grms. of seed with one grm. of bunt powder

The treatments were as follows in both the series:—

(i) Control—untreated seed—naturally infected in series A and a further dose of artificial infection in series B as stated above

(ii) Seed treated with finely powdered copper carbonate at the rate of two ounces per bushel (60 lbs.)

(iii) Seed treated with ceresan at the rate of two ounces per bushel

(iv) Seed treated with formalin solution 0·25 per cent for ten minutes and exposed to formalin vapour for four hours.

(v) Seed treated with uspulun (universal) 0·25 per cent solution for thirty minutes

The experiment was carried out on a piece of land which was laid out on a randomized block system with eight replications. Each block contained thirty plots, each 10 ft. × 5 ft. in size, fifteen under infection series A and fifteen under infection series B. Each plot had five rows of plants. There were five treatments and three varieties in each series.

At the time of harvest all the ears in each plot were counted and the number of ears showing bunted grain was noted down. The total number of healthy and bunted ears in each of the eight blocks under both series of treatments for the three wheat varieties separately is shown in Table VII. The amount of bunt in each plot is given as a percentage of the total number of ears, which is shown in Table VIII.

TABLE VII

The number of healthy and bunted ears in three varieties of wheat

Treatment	Varieties	Block 1		Block 2		Block 3		Block 4		Block 5		Block 6		Block 7		Block 8	
		A		B		A		B		A		B		A		B		A		B	
		H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Control	P 111	530	5	608	45	750	6	550	21	701	6	461	16	825	7	453	17	495	8	390	23	702	4	812	70	720	5	640	21	836	4	463	14
	P 112	517	8	620	31	598	10	636	37	450	24	523	51	550	16	490	50	520	10	611	50	750	20	750	41	640	25	811	27	750	16	640	40
	P 113	480	16	714	16	552	16	570	13	566	18	678	24	650	20	635	16	564	16	605	29	520	11	593	12	805	20	550	16	665	16	720	23
Copper carbonate	P 111	508	2	312	1	511	1	550	1	480	2	586	1	365	1	680	2	380	1	732	3	704	3	850	2	653	8	612	2	324	5	416	2
	P 112	360	2	500	4	572	10	580	2	405	4	462	2	570	5	675	3	557	5	795	3	840	4	839	1	680	4	574	2	535	3	654	4
	P 113	770	1	548	4	587	2	710	2	710	1	722	2	665	0	670	4	540	1	610	4	872	2	1105	1	662	1	600	1	710	1	824	5
Ceresan	P 111	525	1	454	2	440	3	380	3	565	2	716	2	412	1	735	3	549	1	850	3	808	2	865	3	542	3	350	2	610	1	402	2
	P 112	435	4	520	5	521	4	710	6	413	8	550	6	634	10	800	4	542	7	1107	13	725	6	814	2	536	5	695	6	654	9	928	3
	P 113	436	1	480	1	510	2	782	4	532	2	840	1	480	2	690	1	424	1	518	1	654	1	809	2	420	2	530	2	440	1	701	3
Formalin	P 111	450	10	60	0	900	6	84	0	360	4	125	0	370	6	153	0	593	5	224	2	450	4	329	2	320	4	45	0	430	6	51	0
	P 112	460	3	210	1	459	5	600	2	572	4	191	0	470	3	240	6	583	5	360	5	581	5	520	8	620	2	200	2	690	5	158	5
	P 113	708	3	308	1	635	3	365	1	605	5	215	2	531	6	225	1	672	3	302	3	930	5	470	5	470	1	324	0	714	1	225	3
Uspulun (Universal)	P 111	622	1	453	1	520	1	240	1	653	1	540	3	470	0	504	1	850	1	550	0	826	1	650	4	605	2	575	2	471	0	460	1
	P 112	480	2	519	0	620	1	750	2	570	1	667	2	760	6	732	3	736	2	800	4	410	2	870	2	570	6	820	1	527	1	590	2
	P 113	525	7	540	0	460	2	710	6	810	2	645	1	940	2	716	3	830	4	805	5	915	3	1010	8	520	3	480	3	450	2	716	2

H—Total No. of healthy ears ; D—Total No. of bunted ears. A—Series A. B.—Series B.

TABLE VIII

Percentage of infection

Block	Varieties / Infection series	Treatment: Control P111	Control P112	Control P113	Copper carbonate P111	Copper carbonate P112	Copper carbonate P113	Ceresan P111	Ceresan P112	Ceresan P113	Formalin P111	Formalin P112	Formalin P113	Uspulun P111	Uspulun P112	Uspulun P113	Infection total Series A	Infection total Series B	Block totals A+B
1	A	·94	1·54	3·33	·39	·55	·12	·19	·19	·22	2·22	·65	·42	·16	·41	1·33	13·38	—	
	B	7·40	5·00	2·23	·32	·80	·72	·44	·06	·20	·00	·47	·32	·22	·00	·00	—	19·08	32·46
2	A	·80	1·67	2·89	·19	1·74	·34	·68	·76	·39	·66	1·08	·48	·19	·16	·41	12·44	—	
	B	3·81	5·51	2·28	·18	·34	·28	·78	·84	·51	·00	33	·27	·41	·28	·51	—	16·96	29·40
3	A	·85	5·33	3·18	·41	·98	·14	·35	1·93	·37	1·11	·69	·82	·15	·17	·24	16·72	—	
	B	3·47	9·75	3·53	·17	·43	·27	·27	1·09	·11	·00	·00	·93	·55	·29	·15	—	21·01	37·73
4	A	·84	2·90	3·07	·29	·87	·00	·24	1·57	·41	1·62	·63	1·12	·00	·78	·21	14·55	—	
	B	3·75	10·20	2·44	·29	·44	·59	·40	·50	·14	·00	2·50	·44	·19	·40	·41	—	22 69	37·24
5	A	1·64	1·92	2·83	·26	·89	·18	·18	1·22	·23	·84	·85	·44	·11	·27	·43	12·34	—	
	B	5·59	8·18	4·79	·46	·37	·65	·55	1·17	·19	·89	1·38	·99	·00	·50	·62	—	26·37	38·71
6	A	·56	2·66	1·34	·42	·47	·22	·24	·82	·15	·88	·86	·50	12	48	·32	10·04	—	
	B	8·62	5·48	2·02	·23	·11	·09	·34	·24	·24	·60	1·53	1·05	·61	·22	79	—	22·16	52·20
7	A	·69	3·90	2·48	1·22	·58	·15	·55	·93	.47	1·25	·32	·31	·33	1·05	·57	14·70	—	
	B	3·28	3·33	2 90	·32	·34	·16	·57	·56	·37	·00	1·00	·00	·34	·13	·62	—	14·20	28·90
8	A	·47	2·13	2·40	1·54	·56	·14	·16	1·37	·22	1·39	·72	1·40	·00	·18	·44	13·12	—	
	B	3·02	6·25	3·19	·48	·61	·60	·49	·32	·43	·00	3·16	1·33	·21	·33	·27	—	20·68	33·80
Varietal series total	A	6·79	23·05	21·52	4 73	6·64	1·29	2·59	9·51	2 46	9·97	5·80	5·39	1·08	3·51	4·00	107·29	—	
,, ,,	B	39·24	53·07	23 38	2 39	3·44	3 36	3·64	5·98	2..8	1·49	10·37	5·34	2·53	2 14	3·70	—	163·15	270·44
Varietal totals of A+B		46·03	76·02	44·90	7·11	10·08	4·65	6·23	15·49	4·64	11·46	16.17	10·73	3 59	5·64	7·70	107·29	163·15	270·44
Treatment totals		166·95			21·84			26·36			38·36			16 93			270·44		270 44

It will be noted from the tables that infection in series B is heavier than that in series A, that is, the extra dose of infection given in series B has definitely increased the percentage of bunt. It will also be observed that uspulun (universal) though unable to control the disease altogether gave the least percentage of bunted ears.

Statistical analysis of the data is worked out according to Fisher's analysis of variance [1932] as shown in Table IX.

TABLE IX

Analysis of variance

Variance due to	Degrees of freedom	Sum of squares	Mean square	Observed value of Mahalanobis' x	Critical value of Mahalanobis' x_p = ·01)
Block	7	3.305	0.472	..	..
Treatments . . .	4	336.974	84.244	155.144	3.320
(Control and fungicides) Infections (A and B) . .	1	13.002	13.002	23.946	6.635
Varieties (Pusa 111, 112 and 113)	2	20.755	10.378	19.111	4.605
Interactions . . .	22	149.881	6.813	12.547	1.791
Residual	203	110.239	0.543	..	..
Total . .	239	634.156	..	..	..

It will be seen from the above table that the calculated value of x exceeds the theoretical value given in Mahalanobis' table [1932] for one per cent level of significance in all items except blocks showing thereby that the differences between the several treatments, infections and varieties are statistically significant. The differences between the mean values of the different treatments—control, copper carbonate, ceresan, formalin and uspulun (universal) are given in table X.

TABLE X

The differences between the mean values for treatment

Treatments	1 Control	2 Copper carbonate	3 Ceresan	4 For malin	5 Uspulun
Control	..	—3.023	—2.929	—2.679	—3.125
Copper carbonate . .	+3.023	..	+0.094	+0.344	—0.102
Ceresan	+2.929	—0.094	..	0.250	—0.196
Formalin	+2.679	—0.344	—0.250	..	—0.446
Uspulun	+3.125	+0.102	+0.196	+0.446	..
Mean	3.478	0.455	0.549	0.799	0.353

The critical differences for treatments have been calculated and found to be:—

Critical difference=0·304 (P= ·5)

Critical difference=0.401 (P= ·01)

A comparison of the various figures with the critical differences leads to the following conclusions:—

Treatment 5 (uspulun universal) is superior to treatment 4 (formalin) at the one per cent level. Treatment 2 (copper carbonate) is superior to treatment 4 (formalin) at the five per cent level. Treatment 2 (copper carbonate), treatment 3 (ceresan), treatment 4 (formalin) and treatment 5 (uspulun) are superior to treatment 1 (control—undisinfected seed) at the one per cent level. There is no significant difference between other combinations.

The differences between the mean of different varieties *viz.* Pusa 111, 112 and 113 are given in Table XI.

TABLE XI

The differences between the mean values for varieties

Varieties	Pusa 111	Pusa 112	Pusa 113
Pusa 111	..	0.613	—0.022
Pusa 112	—0.613	..	—0.535
Pusa 113	0.022	0.535	..
Mean	0.930	1.543	0.908

Critical difference = .229 (P = .05)

Critical difference = 0.303 (P = ·01)

From the above table it is evident that Pusa 112 is inferior to Pusa 111 = Pusa 113 at the one per cent level in respect of susceptibility to bunt

Comparing the two series of infections we find that

Mean of A = 0·893

Mean of B = 1·360

∴ Mean difference = 0·467

Critical difference = 0.247 (P = ·01)

Thus infection is significantly higher in the B series than in the A series as was expected on account of extra dose of infection given in the former.

In another experiment heavily infected seed of Pusa 72-1-1 was treated and sown in Karnal under the following treatment:—

1. Control—heavily infected seed
2. Seed treated with copper carbonate at the rate of two ounces per bushel
3. Seed treated with ceresan at the rate of two ounces per bushel

At the time of harvesting all the ears were counted and those having bunted grain were also noted. The following data give the percentage of bunted ears.

TABLE XII

The effect of various fungicides on T. indica.

Treatment	No. of ears	No. of ears with bunted grain	Percentage of bunted ears
Control	8535	455	5.33
Copper carbonate	4105	68	1.65
Ceresan	4152	1081	2.60

This experiment also shows that copper carbonate and ceresan are able to reduce the percentage of bunted ears from 5.33 to 1·65 and 2·60 respectively but none of these treatments can completely control the disease. Untreated seed of this variety which produced 5.33 per cent of bunted ear under Karnal conditions was sown at Pusa as explained in Experiment V but produced a healthy crop with no bunted ear.

Microscopic examination of lightly diseased grain shows that the fungus occurs under the seed-coat and that it is well protected by the pericarp, especially in the lightly attacked grain. The fungicides therefore are unable to kill the internal mycelium or the spores, though they are able to reduce the percentage of disease. Hot water treatment may be a possible method to check the disease to a greater extent which will, in addition destroy loose smut (*Ustilago tritici* (Pers.) Jens.). Luthra [1934] has found that dipping the seed in water for four hours and then exposing it to solar heat for four or five hours during summer can check the loose smut. It remains to be seen whether this treatment will check bunt as well. Experiments are being conducted to see the efficacy of the hot water treatment for bunt control and should the method be successful, it will be very useful in controlling both bunt and loose smut by one and the same treatment.

During 1932-33 bunt was very slight or almost absent on Pusa 114 at Karnal and during 1933-34 seed of Pusa 114 treated with ceresan was sown in a plot in which during 1932-33 there was Pusa 111 showing bunt upto an extent of two per cent. An examination of seed of Pusa 114 showed a fair amount of bunt. It appears that the soil was infected from bunt spores fallen from Pusa 111 during 1932-33 and this perhaps may have infected Pusa 114. That infection takes place from soil in other species of Tilletia is well known. It is quite possible that Pusa 114 got infection from the soil infected previously. Thus rotation of crops is also advisable and wheat should not, if possible, be sown on land occupied in the previous year by the same crop.

IX. Conclusion and summary

Bunt on wheat in India is caused by three species of Tilletia, that is, *T. caries*, *T. foetens* and *T. indica* and is confined to the north western parts of India and does a fair amount of damage. *T. caries* and *T. foetens* are restricted to the cooler regions while *T. indica* is confined to the plains. All the three species possess a stinking smell. *T. indica* can very easily be distinguished from other species by its partial attack on the grain and also by its black coloured spore mass, while in the other two species the whole grain except the seed-coat is destroyed and the spores are dusty olive brown or rust coloured in mass. Spores of *T. indica* are much larger than those of *T. caries* to which it is closely allied and it appears that there are at least two physiologic forms of *T. indica*. The biometric analysis of data of spore measurement shows that this method can be employed to determine physiologic forms though it is not enough to identify the different species. There is little possibility of any wind dissemination of spores directly from the bunted head as occurs in the case of *Ustilago tritici* (Pers.) Jens. The bunted grain is wholly or partly concealed by the glumes and before the harvest there is little or no opportunity for the dissemination of the bunt spores. When the crop is mature, the bunted grain is sometimes easily displaced and falls on the ground with a slight disturbance and thus infects the soil. The danger of dissemination of bunt spores lies in the threshing operation when they adhere to the surface of the sound grain and lodge in the brushes. Large number of grains thus get infected. When the bunted grains come in contact with the healthy ones in storage, they infect the latter very easily. It has also been shown by a series of infection experiments done at Karnal and Pusa that infection does not take place in Pusa whereas at Karnal the disease appears almost every year. It is due to different climatic conditions.

Control measures tried show that the percentage of infection can be reduced by treatment with fungicides like uspulun (universal), copper carbonate, ceresan and formalin but none of them is able to check the disease altogether, the reason being that spores are well protected in mildly attacked grain by the pericarp and the fungicide cannot reach the spores. It is supposed that hot water treatment may be a possible method to check the disease to a greater extent, and as loose smut is also very common on wheat, one treatment might do for both the diseases. Further, there is an indication that as in other bunts infection can take place from infected soil and so rotation of crops is advisable. It would be better if from time to time Karnal seed is renewed with seed from Pusa which is free from both bunt and smut.

Acknowledgments

The writer's grateful thanks are due to Dr. F. J. F. Shaw, Director and Imperial Economic Botanist, Pusa, for placing at his disposal land and other facilities at Karnal for carrying on the experiments on control measures. He is also thankful to Mr. R. D. Bose, Offg. Assistant Economic Botanist, Pusa,

for help given in the statistical interpretation of the field data and to Mr. R. B. Ekbote of the Botanical Sub-Station, Karnal, for looking after the experimental plot. Dr. B. B. Mundkur's help is also acknowledged.

REFERENCES

Brentzel, W. E. (1933). *Phytopath.* **23,** 483-485.

Faris, J. A. (1924). *Mycologia* **16,** 259-282.

Fisher, R. A. (1932). 'Statistical Methods for Research Workers'.

Heald, F. D. (1916). *Proc. Wash. State Grain Growers', Shippers' and Millers' Assoc.* 38-45.

Heuser, W. (1922). *Fühling's Landw. Zeit.* **71,** 81-99.

Howard, A. and Howard, G. L. C. (1909). 'Wheat in India'.

Hungerford, C. W. (1922). *Phytopath.* **12,** 337-342.

Luthra, J. C. and Sattar, A. (1934). *Ind. J. Agric. Sci.* **4,** 177-199.

Mahalanobis, P. C. (1932). *Ind. J. Agric. Sci.* **2,** 686-689,

Mitra, M. (1931, 1). *Ann. Appl. Biol.* **18,** 178-179.

Mitra, M. (1931, 2). *Trans. Brit. Myc. Soc.* **16,** 115-127.

McAlpine, D. (1910). 'The Smuts of Australia' Melbourne.

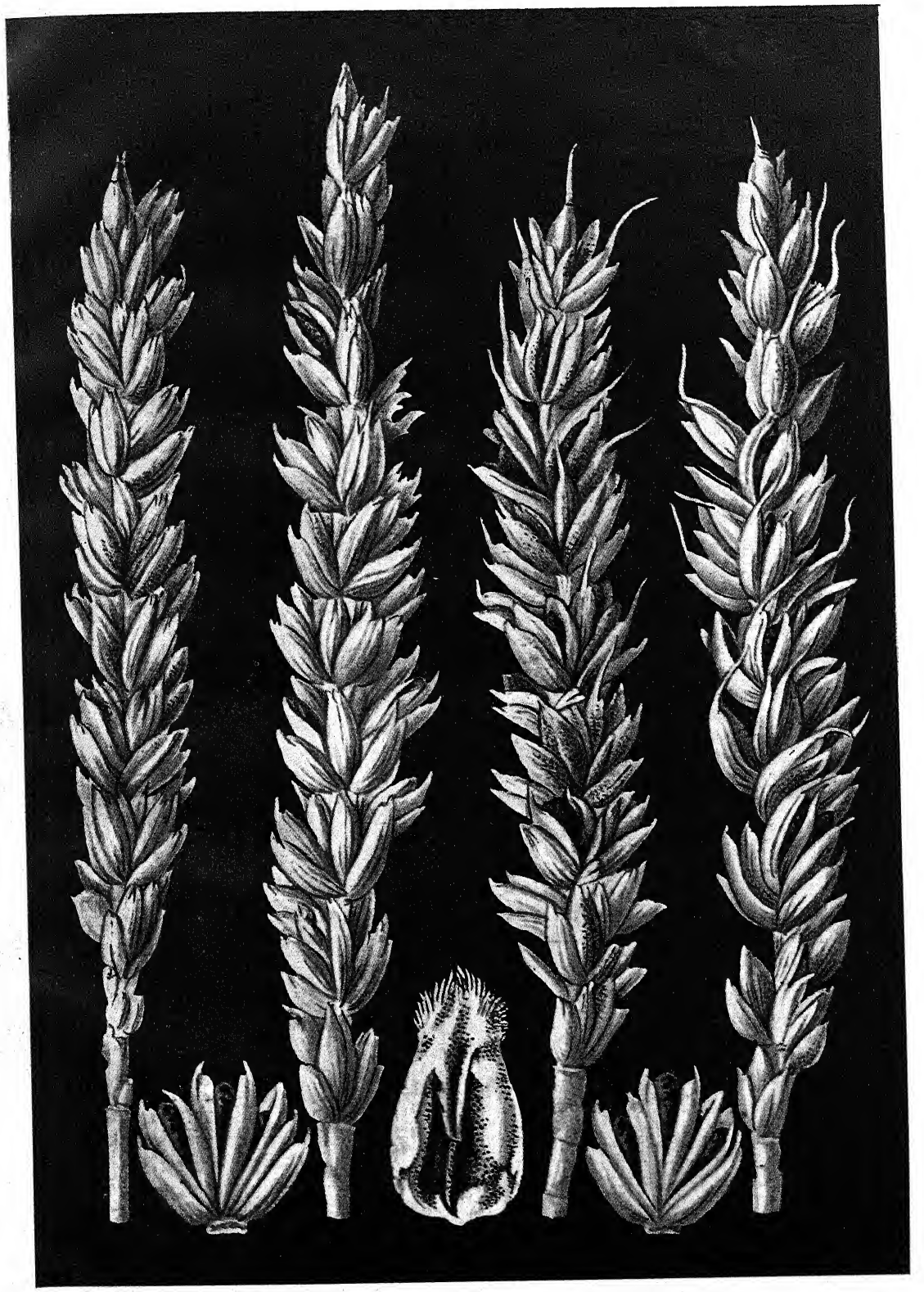
Munerati, O. (1922). *Accad. Lincei. Rend. Cl. Sci. Fis. Mate, Nat.* **31,** 121-29,

———— (1923). *Ibid.* **32,** 285-289.

Stakman (1929). *Proc Int. Cong. Plant Sci, Ithaca,* 1927, **2,** 1312—1330.

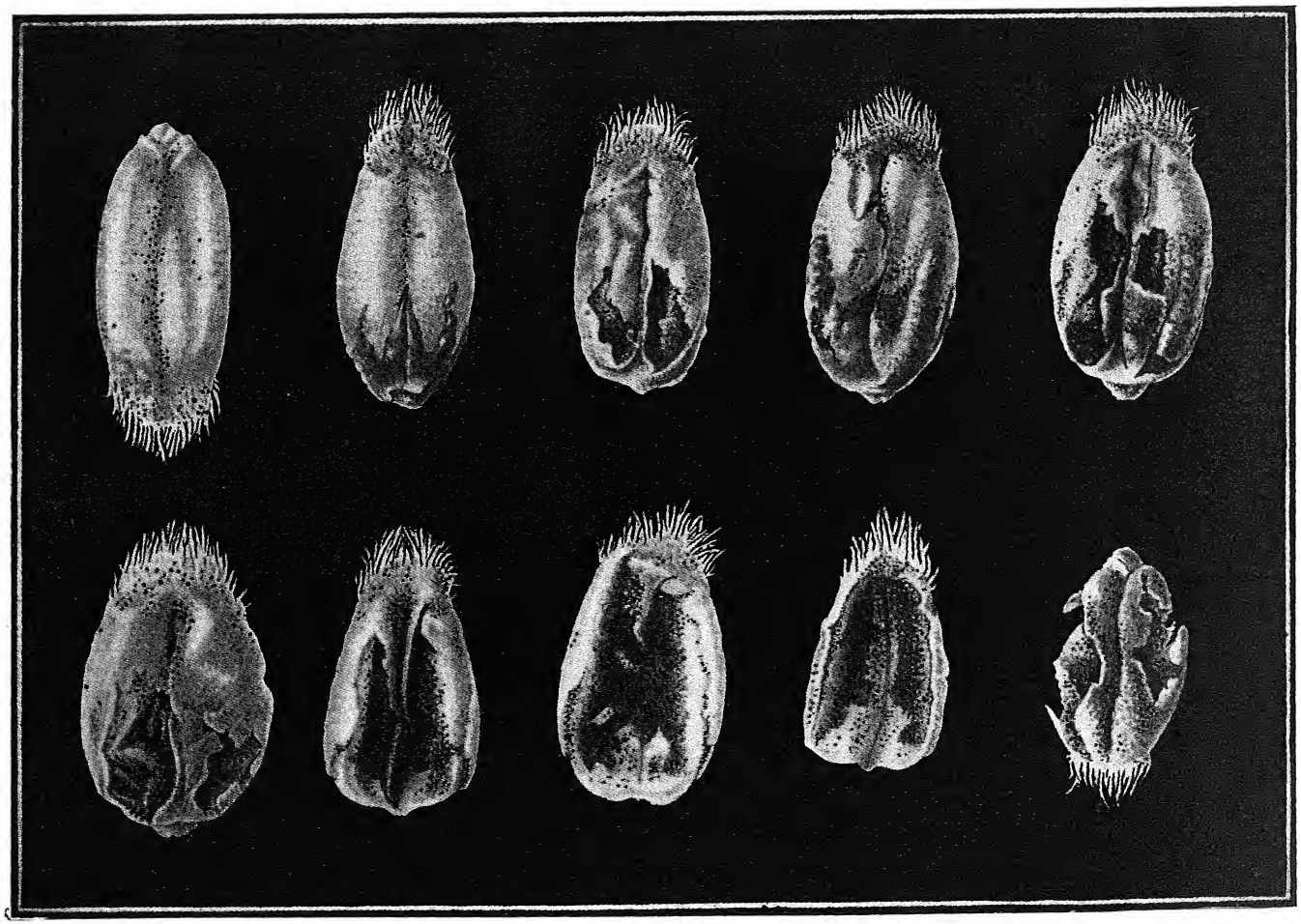
Tisdale, W. H., Leighty, C. E. and Boerner, E. G. (1927). *Phytopath.* **17,** 167-174.

Volkert, A. (1906). *Landw. Jahrb. Schweiz.* **20,** 445-490.



Infected wheat ears. Two spikelets with bunted grain and a heavily infected grain are also shown.

TILLETIA INDICA



Infected wheat grain with various stages of infection from mild to severe.

TILLETIA INDICA

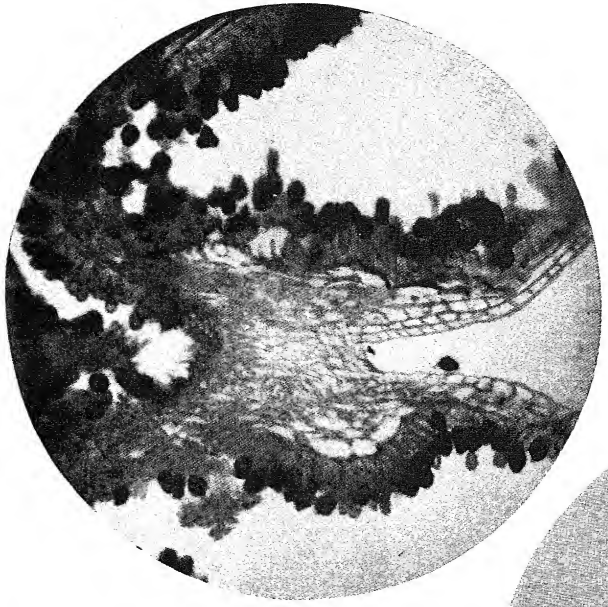


FIG. 1.

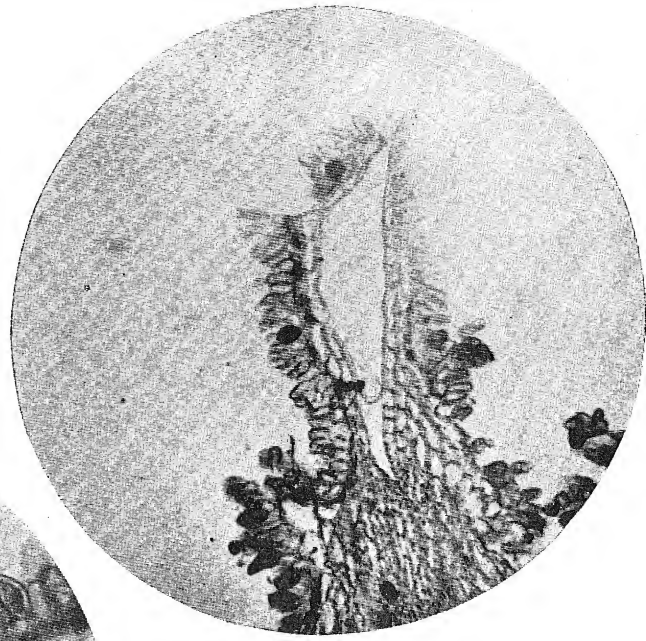


FIG. 2.

FIG. 3.

Photomicrographs of sections of infected grain showing sori and formation of spores (×500).

TILLETIA INDICA

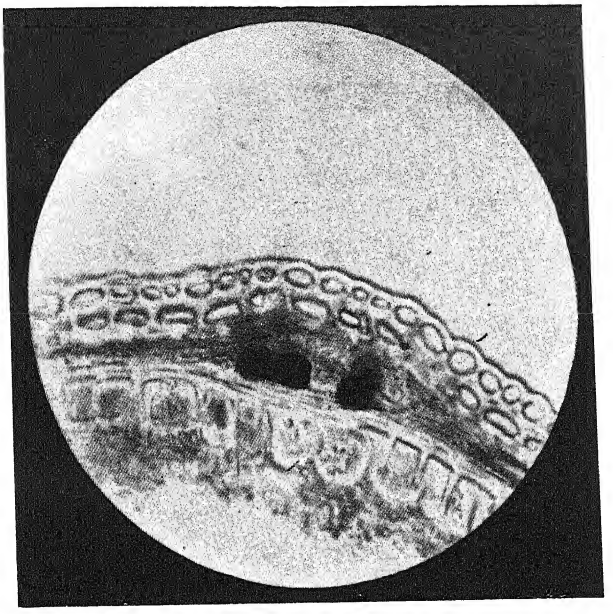


FIG. 1.

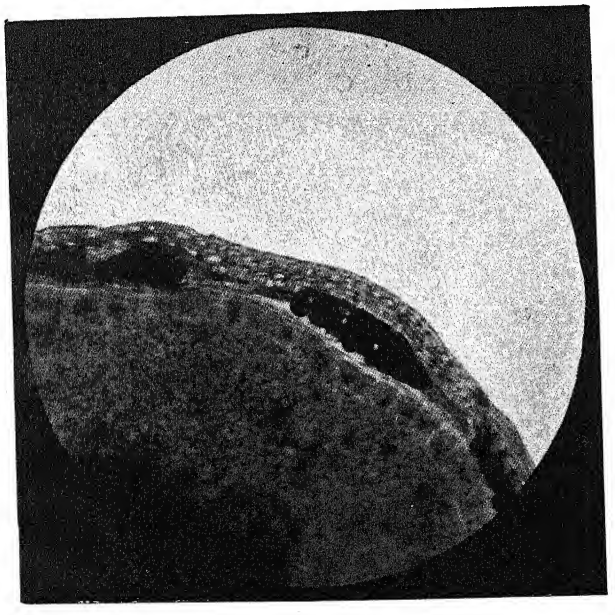


FIG. 2.

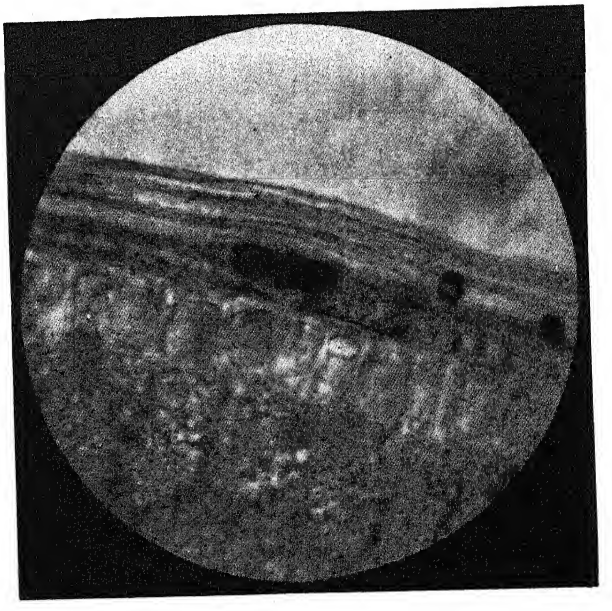


FIG. 3.

Spore formation in lightly infected grain under the seed-coat and above the aleurone layer of endosperm (×500).

TILLETIA INDICA

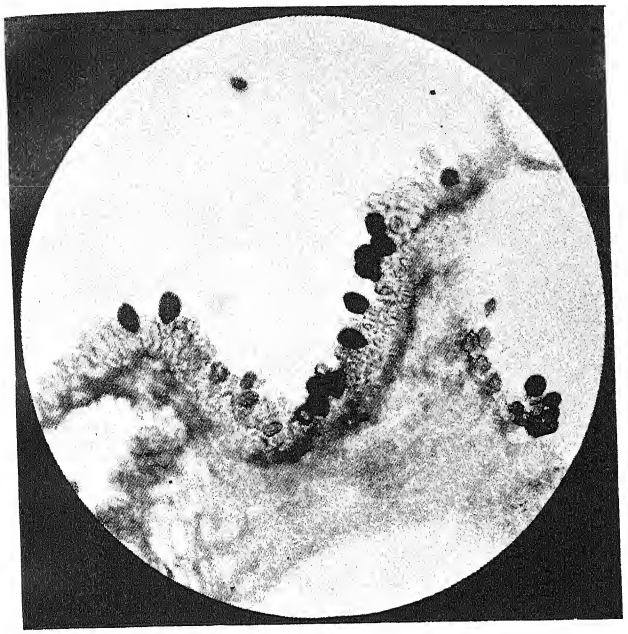


FIG. 1. Spores and sterile cells in sori.

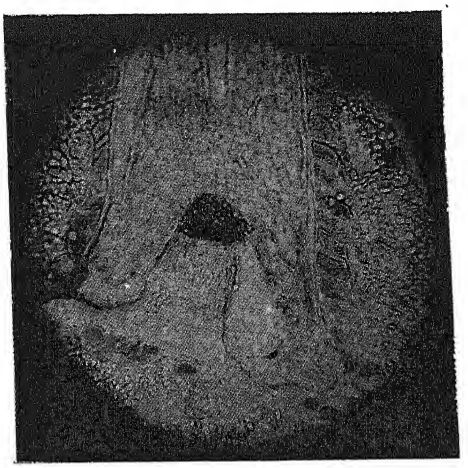


FIG. 2. Formation of a sorus in the tissue on the ventral surface.

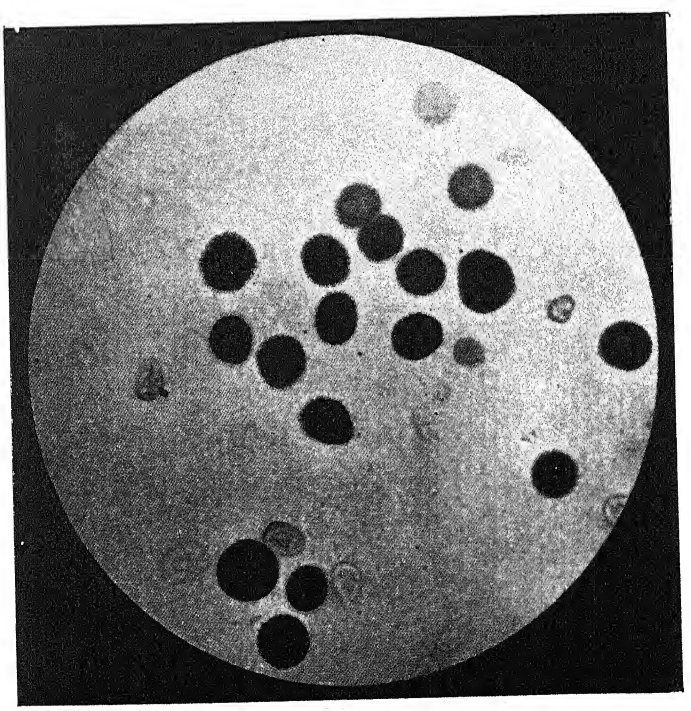


FIG. 3. Mature and young spores with lighter colour sterile cells.

TILLETIA INDICA

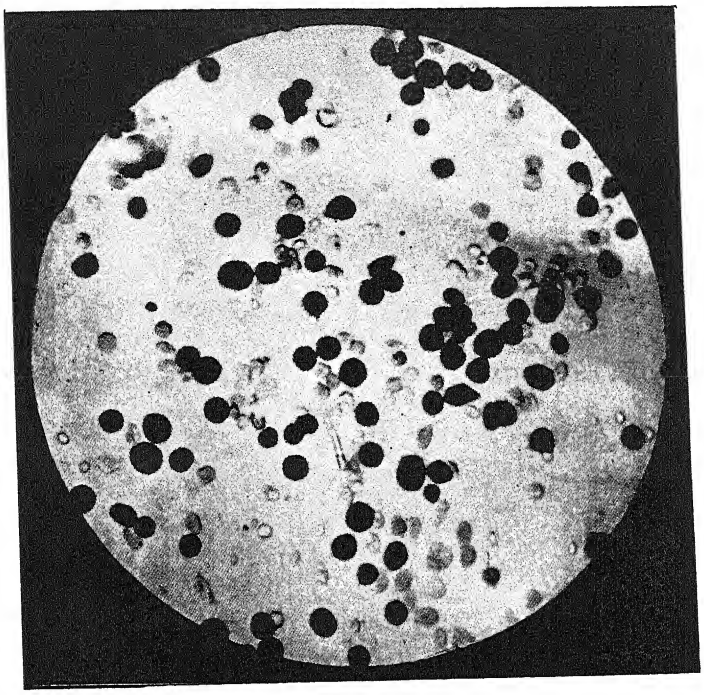


FIG. 1. Mature and young spores with lighter colour sterile cells.

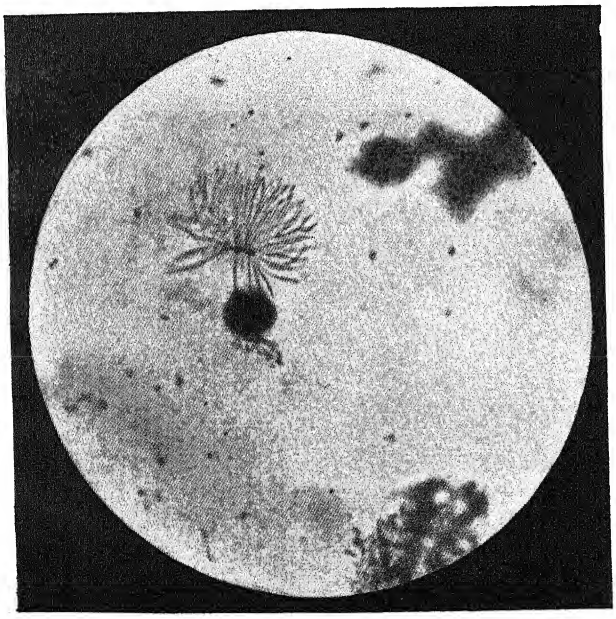


FIG. 2. A germinated spore with a short promycelium bearing a crown of sporidia.

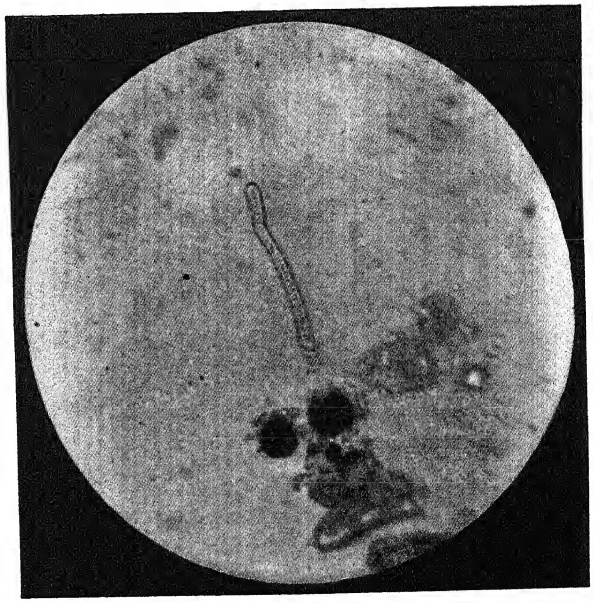
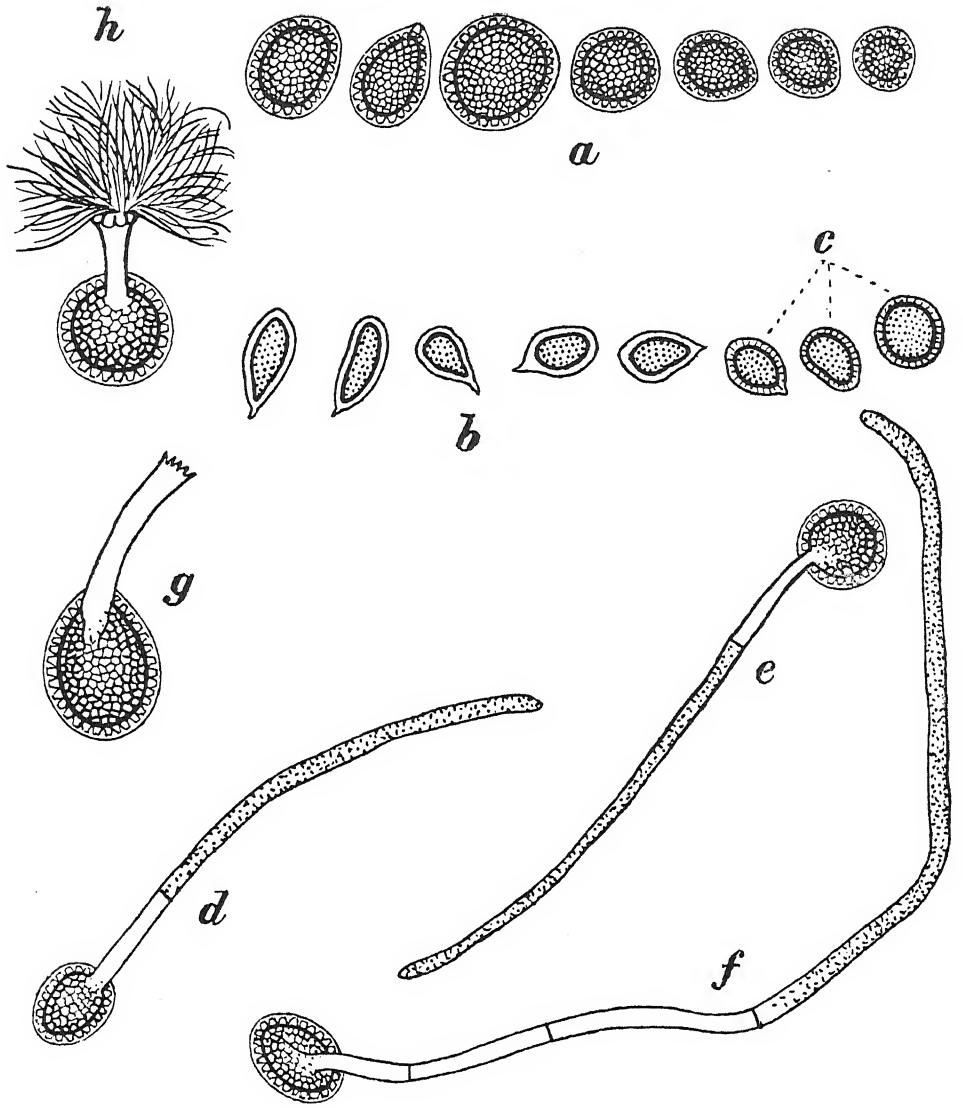


FIG. 3. A germinating spore with a promycelium.

TILLETIA INDICA



(a) Mature spores with reticulation of epispore. One spore with a short papilla (×200).

(b) Sterile cells (×200).

(c) Young spores. One with a papilla (×200).

(d), (e) & (f) Germination and formation of promycelium (×200).

(g) A germinated spore showing the place of attachment of sporodia (×200).

(h) A germinated spore bearing a crown of sporodia (×200).

THE RELATION OF SOME PLANT CHARACTERS TO YIELD IN SORGHUM

BY

G. N. RANGASWAMI AYYANGAR, B. A., I. A. S.,

Millets Specialist, Agricultural Research Institute,

M. A. SANKARA AYYAR, B.A., B.Sc. (Ag).,

P. V. HARIHARAN, B. Sc. (Hons.),

AND

D. S. RAJABHOOSHANAM, M. A.,

Assistants, Millets Breeding Station, Coimbatore.

(Received for publication on 20th September 1934)

The sorghum plant has been under study at the Millets Breeding Station for some years. A number of characters of a qualitative kind have been pursued and in May 1931 a study of the relationship between some of the prominent plant characters and the all-important question of yield was commenced. A rough summary of the results of first attempts was given [Rangaswami Ayyangar, 1932] in the 'Symposium on Sorghum' on the occasion of the Annual Conference of the Madras Agricultural Students' Union in December 1931.

Materials and methods

These studies have been made in three seasons, two of them summer (irrigated) and one main (rain-fed). Five Coimbatore varieties were the subjects of study. They are listed below:—

Season	Strain No.	Name of variety	Colour of grain	Year	Number of plants measured
Summer (irrigated) . .	*A. S.* 809	*Chinna Manjal*	Yellow .	1931	240
				1932	124
	„ 1063	*Uppam Cholam*	White	1931	243
				1932	125
Main (rain-fed) . . .	„ 60	*Peria Manjal*	Yellow (Brown wash).	1931	250
	„ 1098	„	„	1931	250
	„ 129	*Sen Cholam*	Red .	1931	250

All the varieties belong to the group *Sorghum durra* Stapf. The sorghum plant being single-eared the yield is not complicated by tillers as in other cereals. The individual plants were taken from a bulk plot, care being taken to steer clear of diseased individuals and plants at borders. At 'shot blade' stage, plants were labelled and numbered in the field.

The following characters were chosen:—1. Grain yield. 2. Thickness of earhead, 3. Length of earhead, 4. Diameter of peduncle, 5. Weight of dry straw, 6. Weight of 100 grains, 7. Weight of earhead—fresh, 8. Weight of earhead—dry, 9. Length of peduncle, 10. Duration.

The relation of yield to the other characters is, in this study, treated by the method of correlation. The suffix of r is as numbered in the list of characters enumerated above. In Table I are presented the total correlations of grain yield with eight characters (and one more character, *i.e.*, duration, in one season).

TABLE I

Total correlation coefficients

Character correlated with grain yield	Irrigated				Rain-fed 1931 Main		
	A. S. 809		*A. S.* 1063				
	Summer 1931	Summer 1932	Summer 1931	Summer 1932	*A. S.* 60	*A. S.* 1098	*A. S.* 129
Thickness of earhead	.772	.813	.851	.712	.787	.768	.781
Length of earhead	.748	.535	.701	.457	.828	.775	.847
Diameter of peduncle	.845	.739	.879	.569	.870	.855	.857
Weight of dry straw	.588	.451	.702	.375	.763	.828	.835
Weight of 100 grains	.701	.805	.713	.731	.260	.394	.175
Weight of earhead—fresh.	.927	.976	.986	.954	.968	.960	.979
Weight of earhead—dry	.982	.972	.992	.953	.984	.999	.982
Length of peduncle	—.253	—.559	—.097	.119	.203	.157	—.137
Duration	—.685	..	—.601	..	..	..	..
Level of significance	.165	.227	.165	.227	.161	.161	.161
F .01							

As there are seven coefficients for each pair of factors, it is expedient to test whether the same degree of correlation is shown (1) by the same variety in two seasons. (2) in the same season by two varieties, (3) by the irrigated and the rain-fed varieties by themselves, and (4) by all the seven taken together. The test is applied after the hyperbolic transformation $z=\tanh^{-1}r=\frac{1}{2}\{\log_e(1+r)-\log_e(1-r)\}$. In columns 2 to 5 of Table II, the significance of the difference of the z's is measured by considering whether $\frac{Z\sim Z'}{6_{z\sim z'}}$ is greater or less than 2 $\chi^2=S(z-\bar{z})^2(n-3)$ where $\bar{z}$ is the weighted mean of the z's, (the weights being proportional to n-3) and n, the size of the sample. χ_1^2 and χ_2^2 in columns 6 and 7 relate to irrigated and rain-fed varieties respectively; χ^2 combines all the seven coefficients. Further it can easily be shown that χ^2 is greater than $\chi_1^2+\chi_2^2$. Therefore, if $\chi_1^2+\chi_2^2$ exceeds the χ^2 level for $P=\cdot 05$, χ^2 need not be calculated separately. For the details of the technique employed, reference may be made to Tippett's "The Methods of Statistics" [1931]

TABLE II

Combination of the correlation coefficients

Character correlated with grain yield	$\frac{z \sim z^1}{z \sim z^1}$				χ_1^2 Irrigated $\chi_1^2=7\cdot82$ $P=\cdot05$	χ_2^2 Rain-fed $\chi_2^2=5\cdot99$ $P=\cdot05$	χ^2 $\chi^2=12\cdot59$ $P=\cdot05$
	A. S. 809 Summer 1931 and 1932	*A. S.* 1063 Summer 1931 and 1932	Summer 1931 *A. S.* 809 & *A. S.* 1063	Summer 1932 *A. S.* 809 & *A. S.* 1063			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Thickness of earhead .	1·00	3·36	2·53	1·95	12·96	·22	..
Length of earhead .	3·36	3·45	1·10	0·86	29·99	4·40	..
Diameter of peduncle .	2·64	6·55	1·43	2·34	48·72	0·42	..
Weight of dry straw .	1·73	4·36	2·17	0·70	23·38	60·02	..
Weight of 100 grains .	2·18	0·36	0·22	1·64	5·16	7·26	162
Weight of earhead—fresh	5·18	5·55	9·23	2·66	91·27	13·06	..
Weight of earhead—dry	2·00	8·18	4·51	2·11	72·86	>1000	..
Length of peduncle .	3·36	2·00	1·76	5·86	26·75	18·48	..
Duration . . .	..	..	1·56	..	..	..	..

The correlations show a great deal of variation from variety to variety and season to season; this is apparent from columns (2) to (5) of Table II. From column (6) it is evident that in the irrigated varieties, the same degree of correlation exists between grain yield and the weight of 100 grains. In the case of rain-fed varieties, the correlation coefficients for the several varieties for the thickness of earhead are not different; the same can be said in the case of the length of earhead and diameter of peduncle. But when the coefficients for the several varieties are considered together, they show wide range of variability.

That the correlations show a great deal of variation is not surprising. The relation of one character with another, in the complex plant life, is intricately dependent upon a group of other factors tangible and intangible, and changes in these factors caused by varietal and seasonal fluctuations, will be reflected in the original correlations measured. In the present study, wherever the regressions are not far from linearity, partial correlations are employed to estimate the correlations eliminating some characters. Multiple correlations have also been computed to find whether a knowledge of additional factors helps in the prediction of yield.

Instead of the probable error, the level of significance for $P = \cdot 01$ is calculated for every correlation coefficient [Fisher, 1930]*; this is helpful in judging the significance of the values at sight.

Relation between earhead length and thickness, and yield.—The earhead consists of a group of spikes disposed in whorls round a stalk. This stalk varies in length. In the economic varieties of grain sorghums it stops short of reaching the very top of the earhead, and from its apex radiate spikes which fill and give the earhead the full and globular look it has; so that for the purpose of convenient and practical measurement, the length from the base to the tip of the earhead as a whole was preferred to that of merely the rachis.

The thickness of the earhead was measured by placing it across a metre scale and bringing together two rectangular pieces of wood so as to touch the head on either side without pressing it and the distance between the inner faces of the blocks read direct from the scale. This gives the maximum thickness of the panicle. In the varieties studied this maximum thickness was observed to be at about a third of its length from the base.

The earhead thickness and length are highly correlated with yield in all the varieties and in all the seasons. This is as it should be, considering that the length and thickness are the two dimensions determining the volume of the earhead.

---

* The value of the correlation coefficient for the ·01 level of significance is taken as $\frac{t}{\sqrt{n-2+t^2}}$, where n is the number of pairs of observations, and considering that n is large, t is taken as 2·576 corresponding to $n = \infty$.

TABLE III

Partial correlations

Grain yield and thickness of earhead

r	Irrigated				Rain-fed Main 1931		
	A. S. 809		*A. S.* 1063				
	Summer 1931	Summer 1932	Summer 1931	Summer 1932	*A. S.* 60	A. S. 1098	*A. S.* 129
r_{12}	·772	·813	·851	·712	·787	·768	·781
Level of significance ($P = ·01$).	·165	·227	·164	·226	·161	·161	·161
$r_{12·3}$ . . .	·509	·725	·863	·622	·275	·469	·626
$r_{12·4}$ . . .	·351	·532	·493	·523	·356	·528	·645
$r_{12·5}$ . . .	·643	·779	·716	·667	·612	·589	·703
$r_{12·6}$ . . .	·694	·738	·765	·714	·788	·757	·780
Level of significance ($P = ·01$).	·165	·228	·164	·227	·162	·162	·162
$r_{12·34}$ . . .	·316	·564	·652	·551	·212	·371	·615
$r_{12·45}$ . . .	·367	·605	·496	·554	·367	·494	·667

TABLE III—*contd.*

Grain yield and thickness of earhead—(contd.)

	Irrigated				Rain-fed Main 1931		
	A. S. 809		*A. S* 1063				
	Summer 1931	Summer 1932	Summer 1931	Summer 1932	*A. S.* 60	*A. S.* 1098	*A. S.* 129
$r_{12 \cdot 56}$	·517	·309	·614	·527	·611	·591	·706
$r_{12 \cdot 35}$	·455	·764	·815	·630	·282	·402	·653
$r_{12 \cdot 36}$	·395	·464	·787	·487	·216	·440	·627
$r_{12 \cdot 46}$	·278	·360	·429	·376	·341	·526	·647
Level of significance ($P = ·01$).	.165	·229	·164	·228	·162	·162	·162
$r_{12 \cdot 345}$	.319	·642	·659	·588	·228	·401	·646
$r_{12 \cdot 346}$	.215	·297	·340	·378	·147	·348	·621
$r_{12 \cdot 456}$	.284	·255	·426	·394	·354	·499	·673
$r_{12 \cdot 356}$	.298	·267	·784	·454	·220	·383	·659
Level of significance ($P = ·01$).	.166	·230	·165	·229	·162	·162	·162
$r_{12 \cdot 3456}$	.236	·331	·679	·370	·163	·380	·659
Level of significance ($P = ·01$).	.166	·231	·165	·229	·163	·163	·163

TABLE IV

Partial correlations

Grain yield and length of earhead

r	Irrigated				Rain-fed Main 1931		
	A. S. 809		*A. S.* 1063				
	Summer 1931	Summer 1932	Summer 1931	Summer 1932	*A. S.* 60	*A. S.* 1098	*A. S.* 129
r_{13}	·748	·535	·701	·457	·828	·775	·847
Level of significance ($P = ·01$).	·165	·227	·164	·226	·161	·161	·161
$r_{13·2}$ . . .	·437	·045	·726	—·123	·486	·490	·748
$r_{13·4}$ . . .	·206	—·006	·180	—·037	·332	·493	·498
$r_{13·5}$ . . .	·618	·333	·486	·281	·625	·564	·590
$r_{13·6}$ . . .	·747	·747	·711	·613	·851	·788	·855
Level of significance ($P = ·01$).	·165	·228	·164	·227	·162	·162	·162
$r_{13·24}$ . . .	·130	—·058	—·052	—·208	·166	·307	·447
$r_{13·25}$ . . .	·404	·246	·689	—·010	·322	·368	·510
$r_{13·26}$ . . .	·530	·487	·785	·161	·554	—·127	·825

TABLE IV—*contd.*

Grain yield and length of earhead—(contd.)

r	Irrigated				Rain-fed Main 1931		
	A. S. 809		*A. S.* 1063				
	Summer 1931	Summer 1932	Summer 1931	Summer 1932	*A. S.* 60	*A. S.* 1098	*A. S.* 129
$r_{13 \cdot 45}$ . . .	·191	·187	·181	·056	·330	·341	·410
$r_{13 \cdot 46}$ . . .	·303	·421	·325	·060	·393	·541	·512
$r_{13 \cdot 56}$ . . .	·622	·419	·555	·294	·671	·097	·620
Level of significance ($P = ·01$).	·165	·229	·164	·228	·162	·162	·162
$r_{13 \cdot 245}$ . . .	·106	·175	·663	—·100	·774	·137	·355
$r_{13 \cdot 246}$ . . .	·247	·371	·621	—·070	·253	·369	·469
$r_{13 \cdot 456}$ . . .	—·273	·382	·306	·080	·393	·387	·429
$r_{13 \cdot 256}$ . . .	·487	·381	·744	·091	·406	—·606	·670
Level of significance ($P = ·01$).	·166	·230	·165	·229	·162	·162	·162
$r_{13 \cdot 2456}$ . . .	·236	·360	·622	—·029	·243	·286	·515
Level of significance ($P = ·01$).	·166	·231	·165	·229	·163	·163	·163

The partial correlations obtained by eliminating the other factors though considerably reduced, except in *A. S.* 1063 (summer 1931) and *A. S.* 129 (main 1931), are nevertheless significant. That a partial correlation can be none too cautiously interpreted is well illustrated here. If L be the length of the earhead, and 2 t the thickness, the volume of an earhead, considering its shape, can be taken approximately as kt^2L. The grain yield is nearly proportional to this volume. If n be the packing in unit space and w the weight per grain, W, the total grain yield for the earhead is approximately kt^2Lnw. In this expression, n is the only undetermined factor and it may be supposed nearly constant. The low partial correlations are in contradiction of the obvious fact that, when for instance t and w are held constant, the correlation between W and L is perfect. This anomalous result is because the underlying assumption of linear regression is not fulfilled.

Diameter of peduncle and yield

The thickness or diameter of peduncle was measured with a Vernier callipers at a standard distance of 5 cm. below the base of panicle. The peduncle, which is virtually the end internode, does not show the amount of fluctuation in girth which the internodes lower to it, present.

TABLE V

Partial correlations

Grain yield and diameter of peduncle

r	Irrigated				Rain-fed Main 1931		
	A. S. 809		*A. S.* 1063				
	Summer 1931	Summer 1932	Summer 1931	Summer 1932	*A. S.* 60	*A. S.* 1098	*A. S.* 129
r_{14}	·845	·739	·879	·569	·870	·855	·857
Level of significance ($P=·01$) .	·165	·227	·164	·226	·161	·161	·161
$r_{14·2}$ . . .	·617	·202	·611	·063	·664	·726	·776
$r_{14·3}$ . . .	·617	·604	·746	·383	·557	·700	·541
$r_{14·5}$	·760	·732	·742	·497	·659	·613	·577
$r_{14·6}$ . . .	·823	·748	·828	·708	·885	·863	·870
Level of significance ($P=·01$) .	·165	·228	·164	·227	·162	·162	·162
$r_{14·23}$ . . .	·497	·205	·137	·181	·537	·661	·526
$r_{14·25}$ . . .	·614	·500	·681	·247	·466	·524	·516
$r_{14·26}$ . . .	·653	·400	·617	·354	·703	·743	·800

$r_{14 \cdot 35}$ . . .	·586	·704	·830	·430	·415	·511	·386
$r_{14 \cdot 36}$ . . .	·581	·427	·657	·452	·578	·451	·576
$r_{14 \cdot 56}$ . . .	·693	·244	·672	·499	·684	·674	·612
Level of significance ($P = ·01$) .	·165	·229	·164	·228	·162	·162	·162
$r_{14 \cdot 235}$ . . .	·514	·475	·618	·266	·384	·483	·367
$r_{14 \cdot 236}$ . . .	·501	·225	·138	·341	·563	·692	·568
$r_{14 \cdot 356}$ . . .	·479	·161	·539	·428	·426	·051	·429
$r_{14 \cdot 256}$ . . .	·590	·213	·529	·282	·506	·609	·563
Level of significance ($P = ·01$) .	·166	·230	·165	·229	·162	·162	·162
$r_{14 \cdot 2356}$ . . .	·441	·139	·088	·336	·403	—·023	·430
Level of significance ($P = ·01$) .	·166	·231	·165	·229	·163	·163	·163

The diameter of peduncle is a very reliable index to yield [Rangaswami Ayyangar, 1932]. It is needless to add how easy and practical a guide this character is in the choice of seed material for yield.

The correlations of this character with the thickness and the length of the earhead are high. A thick peduncle is the mark of a large earhead. However, no *a priori* form of the dependence of yield on the diameter of the peduncle can be deduced; the correlation is indirectly through the length and thickness of the earhead. Table V shows the effect of eliminating the other factors step by step. The low values are not to be interpreted too literally for reasons already set forth.

Length of peduncle and yield

Varieties of sorghum vary in the length of their peduncles. The length of peduncle was taken to be the distance between the top-most node and the base of the earhead and measured in centimetres. It is seen from Table I that in four cases the correlations are below the level of significance, and that only in the case of *A. S.* 809 in the year 1932 the correlation value is a little high. The regression is however non-linear as is evident from the following analysis:—

A. S. 809. Summer 1932.

Analysis of variance

Variance	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ Log$_e$ (Mean square)
Linear regression . . .	1	340·6	656·8	..
Deviations from regression .	4	72·0	18·0	1·445
Within arrays . . .	118	677·3	5·74	·874
Total .	123	1089·9	..	..

For $Z = ·571$, $P < ·05$

Dry straw weight and yield

After removing the head each stalk was folded up to a bundle about a foot and a half in length, and tied up with a cotton thread. These bundles were placed in wicker baskets and kept out for drying in the sun undisturbed. This minimised the loss due to shedding leaves. The weights of dry stalks were recorded after the bundles were thoroughly dry.

The correlations are higher in rain-fed than in irrigated varieties.

TABLE VI

Partial correlations

Grain yield and weight of dry straw

r	Irrigated				Rain-fed Main 1931		
	A. S. 809		*A. S.* 1063				
	Summer 1931	Summer 1932	Summer 1931	Summer 1932	*A. S.* 60	*A. S.* 1098	*A. S.* 129
r_{15}	·588	·451	·702	·375	·763	·828	·835
Level of significance ($P=·01$) .	·165	·227	·164	·226	·161	·161	·161
$r_{15·2}$ . . .	·225	—·274	·320	—·175	·559	·706	·778
$r_{15·3}$ . . .	·286	·091	·489	·004	·436	·681	·549
$r_{15·4}$ . . .	—·183	—·429	—·011	—·208	·170	·520	·489
$r_{15·6}$ . . .	·617	·749	·655	·580	·781	·826	·838
Level of significance ($P=·01$) .	·165	·228	·164	·227	·162	·162	·162
$r_{15·23}$ . . .	·133	·359	—·053	—·126	·439	·651	·584
$r_{15·24}$ . . .	—·215	—·526	—·062	—·293	·194	·484	·526

$r_{15 \cdot 26}$ . . .	·355	·387	·375	·146	·595	·717	·788
$r_{15 \cdot 34}$ . . .	·050	—·461	—·020	—·212	·165	·472	·399
$r_{15 \cdot 36}$ . . .	·374	·428	·448	·171	·469	·741	·554
$r_{15 \cdot 46}$ . . .	—·045	·251	·061	—·034	·200	·541	·485
Level of significance ($P = \cdot 01$) .	·165	·229	·164	·228	·162	·162	·162
$r_{15 \cdot 234}$ . . .	—·067	—·558	—·125	—·233	·154	·463	·458
$r_{15 \cdot 236}$ . . .	·269	·218	—·014	·060	·470	·829	·523
$r_{15 \cdot 346}$ . . .	—·008	·164	—·045	—·064	·200	·500	·511
$r_{15 \cdot 246}$ . . .	—·093	·105	·013	—·116	·223	·517	·529
Level of significance ($P = \cdot 01$) .	·166	·230	·165	·229	·162	·162	·162
$r_{15 \cdot 2346}$ . . .	—·142	·046	—·055	—·098	·212	·475	·567
Level of significance ($P = \cdot 01$) .	·166	·231	·165	·229	·163	·163	·163

In the absence of more knowledge of the physiology of the sorghum plant the connection between straw weight and grain yield cannot be established by mere reasoning. Nevertheless it may be argued that the apparent relation between grain yield and straw can be traced to the common factor, the diameter of peduncle. A thick peduncle is the index of a thick stalk and generally of broad leaves, and therefore there is a high correlation between the diameter of the peduncle and the weight of straw ($r_{45} = \cdot 77$ to $\cdot 83$). The partial correlation coefficients, r_{15} ·2346 in the irrigated varieties, are all not significant, and in the rain-fed varieties, they are considerably high, showing that in the varieties growing under dry conditions, straw is a factor to be reckoned with, independent of the other important factors.

Weight of 100 grains and yield

After the heads were threshed, 100 grains picked at random were weighed in grammes. This method was adopted to estimate the weight per grain, because of the impracticability of counting out all the grains in a head.

TABLE VII

Partial correlations

Grain yield and weight of 100 grains

r	Irrigated				Rain-fed Main 1931		
	A. S. 809		*A. S.* 1063				
	Summer 1931	Summer 1932	Summer 1931	Summer 1932	*A. S.* 60[1]	*A. S.* 1098	*A. S.* 129
r_{16}	·701	·805	·713	·731	·260	·394	·175
Level of significance ($P=·01$)	·165	·227	·164	·226	·161	·161	·161
$r_{16·2}$	·589	·726	·510	·733	·267	·348	·160
$r_{16·3}$	·701	·884	·722	·795	·428	·446	·281
$r_{16·4}$	·653	·811	·567	·810	·413	·443	·336
$r_{16·5}$	·720	·898	·668	·800	·358	·380	·221
Level of significance ($P=·01$)	·165	·228	·164	·227	·162	·162	·162
$r_{16·23}$	·648	·799	·633	·736	·397	·415	·283
$r_{16·24}$	·630	·766	·518	·770	·401	·441	·342

TABLE VII—*contd.*

Grain yield and weight of 100 grains—(contd.)

r	Irrigated				Rain-fed Main 1931		
	A. S. 809		*A. S.* 1063				
	Summer 1931	Summer 1932	Summer 1931	Summer 1932	*A. S.* 60	*A. S.* 1098	*A. S.* 129
$r_{16 \cdot 25}$ . . .	·631	·745	·553	·730	·357	·385	·238
$r_{16 \cdot 34}$ . . .	·675	·898	·610	·811	·460	·499	·360
$r_{16 \cdot 35}$ . . .	·723	·906	·706	·802	·462	·456	·321
$r_{16 \cdot 45}$ . . .	·638	·780	·569	·801	·424	·450	·330
Level of significance ($P = \cdot 01$) .	·165	·229	·164	·228	·162	·162	·162
$r_{16 \cdot 234}$ . . .	·651	·801	·633	·750	·438	·483	·372
$r_{16 \cdot 235}$ . . .	·672	·774	·651	·732	·432	·441	·339
$r_{16 \cdot 245}$ . . .	·609	·650	·468	·749	·414	·445	·349
$r_{16 \cdot 345}$ . . .	·529	·783	·673	·878	·471	·513	·354
Level of significance ($P = \cdot 01$) .	·166	·230	·165	·229	·162	·162	·162
$r_{16 \cdot 2345}$ . . .	·494	·644	·719	·853	·429	·504	·376
Level of significance ($P = \cdot 01$) .	·166	·231	·165	·229	·163	·163	·163

The irrigated varieties show a higher correlation than the rain-fed varieties. From the fact that in the former case the regressions are not far from linearity. the high correlations suggest a constancy of the number of grains per earhead; had the correlations been higher, this inference will be very conclusive. The partial correlations are high after eliminating all the other factors, showing that in selection, especially in the irrigated varieties, some consideration to the weight of 100 grains is worthwhile.

Duration and yield

Individuals in a variety having been noted to vary in the time of their flowering to within as much as a fortnight, an attempt was made to relate this difference to yield. Duration was calculated in days from sowing to the commencement of anthesis in the head concerned; the sowing date is more definite than the date of germination. This was recorded in two irrigated varieties for one season.

Within a variety, correlation between duration and yield of grain is negative and high (Table I), i.e., with the increase in duration there is a corresponding decrease in yield. This points to the desirability of compressing if possible the range of flowering period. Moreover, in selection work, there is no need to wait for the late plants; the earlier ones are likely to be better yielders than those delaying.

Duration is very important in an irrigated variety and it is not so great a limitation with a rain-fed variety. For this reason, duration was not considered in the rain-fed varieties.

Weight of earhead, fresh and dry, and yield

The weight of the earhead includes the weight of the standard 5 cm. stalk with which it was cut. The weight of the fresh head was recorded soon after cutting and the heads were kept for drying and their dry weights recorded after they were thoroughly dried for a number of days, special care being taken to avoid the shedding of grain.

Table I shows that the correlations between grain yield and the weight of earhead, fresh and dry, give the highest values. The differences in values between fresh and dry weights are negligible. It would thus be obvious that the weight of the earhead is a sufficiently reliable index of grain weight.

Table VIII presents the multiple correlation coefficients of grain yield with the other characters, beginning with $r_{1.4}$ which is of primary importance because of its high value, and the practicability of considering the diameter of peduncle in actual selection work.

TABLE VIII

Multiple correlations

R	Irrigated				Rain-fed Main 1931		
	A.S. 809		*A. S.* 1063				
	Summer 1931	Summer 1932	Summer 1931	Summer 1932	*A. S.* 60	*A. S.* 1098	*A. S.* 129
r_{14}	·845	·739	·879	·569	·870	·855	·857
Level of significance ($P = ·01$) .	·165	·227	·164	·226	·161	·161	·161
$R_{1 \cdot 24}$ . . .	·866	·824	·910	·714	·887	·892	·916
Level of significance ($P = ·01$) .	·193	·266	·192	·265	·190	·190	·190
$R_{1 \cdot 234}$ . . .	·868	·824	·934	·722	·890	·904	·934
Level of significance ($P = ·01$) .	·214	·294	·213	·293	·210	·210	·210
$R_{1 \cdot 2345}$ . . .	·874	·896	·935	·746	·890	·924	·947
$R_{1 \cdot 2346}$ . . .	·900	·943	·986	·948	·912	·937	·943
Level of significance ($P = ·01$) .	·231	·317	·230	·316	·227	·227	·227

The addition of variates increases R, in some cases significantly and in some cases not. However, throughout ${}^{R}1{\cdot}2345$ and ${}^{R}1{\cdot}2346$ are significantly above ${}^{r}14$. The tests of significance presented in Table IX were carried out by considering P corresponding to ${}^{z}1{\cdot}234p - 14 = \frac{1}{2}\log \frac{({}^{R^2}1{\cdot}234p - {}^{r^2}14)\ (N-5}{3(1-{}^{R^2}1{\cdot}234p)}$ ($p=5, 6$) for degrees of freedom $n_1=3$ and $n_2=N-5$ where N is the size of the samples [Tippett, 1931]. ${}^{R}1{\cdot}2346$ tends to be higher than ${}^{R}1{\cdot}2345$, thus indicating that given ${}^{R}1{\cdot}234$, the weight of 100 grains is more informative than the weight of straw which is less easy to handle. It may be said, that the additional factors, *viz.*, the length and thickness of earhead, and the weight of 100 grains, help to predict grain yeild with a greater precision than is possible with a knowledge of the diameter of peduncle only.

TABLE IX

Tests of significance

Strain	Season	$z_1 \cdot 2345—14$	Degrees of freedom		P	$z_1 \cdot 2346—14$	Degrees of freedom		P
			n_1	n_2			n	n_2	
A. S. 809 . .	Summer 1931 .	1·403	3	235	$<\cdot 01$	1·730	3	235	$<\cdot 01$
A. S. 809 . .	Summer 1932 .	1·972	3	119	$<\cdot 01$	2·406	3	119	$<\cdot 01$
A. S. 1063. .	Summer 1931 .	2·080	3	238	$<\cdot 01$	3·172	3	238	$<\cdot 01$
A. S. 1063. .	Summer 1932 .	1·522	3	120	$<\cdot 01$	2·713	3	120	$<\cdot 01$
A. S. 60 . .	Main 1931 . .	1·313	3	245	$<\cdot 01$	1·796	3	245	$<\cdot 01$
A. S. 1098. .	Main 1931 . .	2·114	3	245	$<\cdot 01$	2·294	3	245	$<\cdot 01$
A. S. 129. .	Main 1931 . .	2·109	3	245	$<\cdot 01$	2·369	3	245	$<\cdot 01$

Review of previous work

The above correlation studies made through three seasons on the Coimbatore varieties, both dry and irrigated, lead us to a review of previous work in this field. So far, correlation studies in Indian sorghums have been reported only in a few Bombay varieties. Patel [1923] tentatively characterised an ideal *jowar* plant as having a large number of nodes and thus a corresponding increase in the number and total leaf surface per plant. The plant must be tall with the top five internodes short, and having a compact earhead and increased number of seeds per spike. Each of these characters bears a high degree of correlation to grain yield. Kottur and Chavan [1927, 1928] determined the correlations between yield and seven other characters and found that the correlations are positive and reliable in five, *viz.*, height of plant, number of leaves, length of rachis, breadth and weight of the ripe ear, and small and unreliable in two cases, *viz.*, length of peduncle and size of the grain. Patel and Patel [1927-28] working on Surat *jowars* arrived at the same conclusion as above.

In the Texas Stations Report for 1928, the following observations occur:— "Correlation coefficients determined in Blackhull Kafir indicated that length of head, length of seed branches, diameter of plants, and weight of green forage are the characters contributing significantly towards production of grain yield." Harper and others [1931] report from Oklahoma that "inheritance studies with sorghum indicated that length of seed branches and the circumference of heads might be associated closely with high yields, while the length of the stalk had little to do with the grain yield." Unlike the above workers Martin [1928] who has reported on plant characters and acreage yields of grain sorghums concludes among other things that "the yields of fields of grain sorghums are more closely correlated with the number of heads per acre than with the size of head or weight of grain per head. The correlation between the number of heads per acre and both the weight per bushel of grain and the average size of heads is either negative or not significant in the three varieties studied. The height of stalks within a given variety is highly correlated with grain yields. The yields of grain and stover in Kafir are closely correlated. The size or plumpness of seeds of a given variety is correlated with weight per bushel."

In our studies, we determined the relationship of nine characters with grain yield. Our results in general agree with those of the Bombay workers for all the characters common to our studies. A feature of our work is an attempt at gauging the comparative behaviour of rain-fed and irrigated varieties grown under different agronomic conditions. The greater elaboration with which these relationships were pursued have now enabled us, armed with that knowledge, to safely dispense with certain of them and seek in the pursuit of a few significant plant characters, valuable aids to yield.

Summary

The correlation between grain yield per plant and eight other plant characters have been determined in two irrigated and three rain-fed Coimbatore varieties of sorghum.

The diameter of peduncle, weight, length and thickness of earhead and straw weight have given high positive correlation values. These characters can be used as reliable indices in selecting for high yield.

The weight of 100 grains has given high correlation values in the irrigated varieties while in the three dry varieties it was low.

The length of peduncle is either not correlated or is negatively correlated with yield.

In the two irrigated varieties studied the duration was found to be negatively correlated with yield.

Partial and multiple correlations were also calculated. The total grain yield of a plant can be predicted very closely, when the diameter of peduncle, length and thickness of earhead, and the weight of 100 grains are all known.

REFERENCES

Fisher, R. A. (1930). Statistical Methods for Research Workers.

Harper, H. J. and others (1931). 'Field Crop Research in Oklahoma'.. (Abstract in *Expt. Stn. Rec.* **64**, 732-733.)

Kottur, G. L. and Chavan, V. M. (1927). *Poona Agric. Coll. Mag.* **19**, 25-29.

———— (1928). *Bombay Dept. Agric. Bull.* No. 151.

Martin, J. H. (1928). *J. Amer. Soc. Agron.* **20**, 1177-1182.

Patel, G. B. (1923). *Poona Agric. Coll. Mag.* **15**, 116-117.

Patel, M. L. and Patel, G. B. (1927-28). *Mem. Dept. Agric. Ind. Bot. Ser* **16**, 1-57

Rangaswami Ayyangar, G. N. (1932). *Madras Agric. J.* **20**, 18-27.

Texas Stations Report (1928). Abstract in *Expt. Stn. Rec.* **62**, 627 (1930).

Tippett, L. H. C. (1931). The Methods of Statistics'. William and Norgate, Ltd., London.

ABSTRACT

Heterosis as a factor in the increase of tobacco yield. 633·71 :575·125

E. A. Osadchuk. (*Junzo-bereznoc Otdelenie V. I. R'a Gosudarstennyi Nikitskii Botaniceskii sad, Nikita Botanic Garden, 1934: Bull. No. 12, pp. 40*) (*Translation of full summary of the paper, issued by the Imperial Bureau of Plant Genetics, School of Agriculture, Cambridge, England*).

The F_1 hybrids of a number of parental combinations were compared with the parents in two successive years, 1929 and 1930 (Table 3, pages 102 and 103).

Not by any means all combinations shewed heterosis; hybrids of varieties genetically closely allied were invariably intermediate, those from unrelated varieties generally shewed at least an increase in height.

Descriptions are given of the varieties used as parents, followed by tables shewing the yields of the parent varieties and F_1 hybrids. The best combination, Nikitskii Dubec 44 × Sumatra Petioled, gave an increase of 35·1 per cent over the better of the two parents, the next best American 572 × Sumatra Petioled, 26·9 per cent; each of these crosses involved (Table 7, page 104) a Sumatra tobacco and a local strain. Two out of the seven combinations gave negative results, *i.e.*, reduced yields.

The same hybrids were grown on a second plot where the soil fertility was much lower and none of them shewed any marked yield increases: the best combination in the first plot here gave a yield intermediate between the parents and the second best shewed a decrease of 11·7 per cent in place of a 26·9 per cent increase. It is evident from this that it is necessary to test the hybrids under a variety of conditions before drawing conclusions regarding heterosis and to do this a system of tests at a series of different centres, analogous to the State variety testing systems, is regarded as necessary.

Similar studies were made on the number of leaves fit for picking. This varied very little in any one variety and the hybrids were mostly intermediate. The two most highly yielding hybrids referred to above had a greater number of leaves than the parents in both plots. The middle leaf of (Table 8, page 105) 50 plants of each type was measured, the length, breadth, area, form (length: breadth) and thickness of the leaves being determined (Table 9, page 106). The leaf area exceeded that of the parents in several of the hybrids, in one case by 17 4 per cent. American 572 × Sumatra Petioled had actually smaller leaves though their number was greater. Only one combination was intermediate in leaf area.

The yields estimated by multiplying the number, size and thickness of the leaves in most cases corresponded fairly closely to the actual yields and this is suggested as a useful method of making yield comparisons, especially in experiments where only one variety is in use.

The yields of first, second and third quality product and degustation tests shewed that though the differences between the varieties were not great, many of the hybrids excelled their parents and the superiority was most pronounced in the combinations shewing most increase in yield. In view of this fact and the ease of effecting the crosses the use of first generation hybrids on a much greater scale is recommended. It is calculated that one trained worker can produce enough seed to sow one ha. in two working days.

TABLE 3

Results of investigation of the F_1 hybrids in comparison with their parent forms

Parent forms Combination	Percentage increase in 1929				Percentage increase in 1930			
	Height		Number of leaves		Height		Number of leaves	
	In comparison with mean yield of parents	In comparison with the higher yielding parent	In comparison with mean yield of parents	In comparison with the higher yielding parent	In comparison with mean yield of parents	In comparison with the higher yielding parent	In comparison with mean yield of parents	In comparison with the higher yielding parent
1. American 572 × Sumatra Petioled	+27	+20	−3	−15	+6	+6	+8	0
2. Dubec Autskii 576 × Havana	+15	+15	+27	+11	+14	+4	+11	+3
3. Dubec Nikitskii 44 × Sumatra Petioled	+16	+6	+9	−5	+7	—4	0	—6
4. Dubec Nikitskii 44 × American 572	+34	+6	+3	−5	+22	+9	+4	−5
5. Deli × Havana	+13	+4	+4	+2	+30	+30	+12	+10
6. Havana × Ameraicn 572	+16	+3	+10	−3	+17	+12	+12	+4
7. Aya Suluk × Havana	+12	+1	+21	+3	+2	−8	−9	−27
8. Dubec Derekoiskii × Havana	+13	+8	+3	−4	+11	+4	0	−19

Parent forms Combination	Percentage increase in 1929				Percentage increase in 1930			
	Height		Number of leaves		Height		Number of leaves	
	In comparison with mean yield of parents	In comparison with the higher yielding parent	In comparison with mean yield of parents	In comparison with the higher yielding parent	In comparison with mean yield of parents	In comparison with the higher yielding parent	In comparison with mean yield of parents	In comparison with the higher yielding parent
9. Sumatra Petioled × Varatik	+13	0	+4	−4	0	−15	−8	−18
10. Persicăn × Dubec Nikitskii 44	+28	−1	+8	−9	+6	−7	+3	−14
11. Ksanti × Dubec Derekoiskii	+6	−3	−3	−11	..	..	..	..
12. Sandre × Dubec Nikitskii 44	+1	−4	+7	−6	−12	−10	+6	−13
13. Kizildza Mursal × Dubec Nikitskii 44	−3	−12	−11	−26	+10	−3	0	−7
14. Havana × Dubec Gurzufskii 282	−1	−14	0	−20	+7	−17	−7	−20
15. Havana × Dubec Nikitskii 44	+15	−9	+7	−11	+18	+3	−8	−12
16. Varatik × Bektemiz	+2	−20	−11	−27	−20	−37	−13	−43
17. Ksanti × Havana	..	−3	..	..	+22	+12	+15	+11
18. American 572 × Aya Suluk	..	..	..	..	+11	+7	−7	−10
19. Dubec Gaspra × American 572	..	..	..	..	+5	5	−3	−9
20. Dubec Gurzufskii × Sansun 84	..	..	..	..	0	−10	−10	−23
21. Havana × Varatik	..	..	..	..	0	−10	−14	−14

TABLE 7

Yield of air-dried leaves in centners per hectare

Name of varieties and combinations	$M+m$	P	Percentage increase	
			Over the mean	Over the higher parent
Plot 1				
Deli	20·3±0·52	2·6	..	..
F_1 Deli × Havana	18·8±0·64	3·4	−1·6	−7·4
Havana	12·2±0·69	5·7	..	..
F_1 Dubec Autskii 576 × Havana	14·5±0·67	4·6	+1·0	−2·0
Dubec Autskii 576	14·8±0·49	3·3	..	..
Dubec Nikitskii 44	14·5±0·41	2·8	..	..
F_1 Havana × Dubec Nikitskii 44	16·4±0·44	2·7	+23·3	+13·1
F_1 Dubec Nikitskii 44 × American 572	19·9±0·66	3·3	+24·5	+16·4
F_1 Havana × American 572	19·6±0·55	2·8	+34·2	+14·6
American 572	17·1±0·85	2·1	..	..
F_1 American 572 × Sumatra Petioled	21·7±0·49	2·3	+33·1	+26·9
F_1 Dubec Nikitskii 44 × Sumatra Petioled	21·1±0·93	4·4	+40·7	+36·1
Sumatra Petioled	15·5±0·55	3·6	..	..
Plot 2				
Deli	14·8±0·42	2·8	..	..
F_1 Deli × Havana	13·8±0·94	6·8	+2·5	−0·66
Havana	12·2±0·50	4·1	..	..
F_1 Dubec Autskii 576 × Havana	13·6±0·69	5·1	+4·7	+2·3
Dubec Autskii 576	13·3±0·77	5·8	..	..
Dubec Nikitskii 44	15·4±0·42	2·7	..	..
F_1 Havana × Dubec Nikitskii 44	12·4±0·72	5·8	+1·0	−1·9
F_1 Dubec Nikitskii 44 × American 572	17·3±0·99	5·7	+1·1	+0·7
F_1 Havana × American 572	17·8±0·75	4·2	+26·2	+1·1
American 572	16·1±0·82	5·4	..	..
F_1 American 572 × Sumatra Petioled	14·2±0·74	5·2	−9·5	−11·7
F_1 Dubec Nikitskii 44 × Sumatra Petioled	15·6±0·71	4·6	+0·13	+0·13
Sumatra Petioled	15·4±0·36	2·3	..	..

TABLE 8

Mean number of leaves picked per plant

Varieties and combinations	Plot 1			Plot 2		
	Total number of leaves	Number of leaves picked per plant	Mean number of leaves per plant	Total number of leaves	Number of leaves picked per plant	Mean number of leaves per plant
Deli . . .	13725	909	15·1	13574	911	14·9
F_1 Deli × Havana .	13379	904	14·8	13890	926	15·0
Havana . .	11986	908	13·3	11164	886	12·6
F_1 Dubec Autskii 576 × Havana	19367	905	21·4	18444	891	20·6
Dubec Autskii 576	20498	907	22·6	18339	899	20·4
Dubec Nikitskii 44	22213	885	23·1	20114	890	22·6
F_1 Havana × Dubec Nikitskii 44	21382	906	23·6	20384	910	22·4
F_1 Dubec Nikitskii 44 × American 572	20996	915	23·2	19276	905	21·3
F_1 Havana × American 572	15305	911	16·8	14667	906	16·2
American 572 .	14579	889	16·4	12879	810	15·4
F_1 American 572 × Sumatra Petioled	20648	840	23·2	18931	919	20·6
F_1 Dubec Nikitskii 44 × Sumatra Petioled	23135	883	26·2	23025	921	25·0
Sumatra Petioled .	15901	910	17·4	15205	916	16·6

TABLE 9

Mean data on dimensions of the middle leaf in the forms investigated

1	Name of varieties and combinations 2	Area in sq. cm. 3	Ratio of length to breadth 4	Mean weight of unit area of leaf 5	Yield calculated from size and thickness of leaf 6	Percentage increase on higher yielding forms 7	Percentage increase on higher yielding forms by calculation 8	Difference between columns 7 and 8 9
	Deli	197	2·27	196	11837	..	..	..
F_1	Deli × Havana	212	2·43	316	12594	+6	−7·4	1·4
	Havana	132	3·28	320	5153	..	..	..
F_1	Dubec Autskii 576 × Havana	148	2·37	391	6034	−7	−2·0	5·0
	Dubec Autskii 576	123	2·19	233	6478	..	..	..
	Dubec Nikitskii 44	114	2·48	246	4066	..	..	..
F_1	Havana × Dubec Nikitskii 44	165	2·46	315	8523	+11	+13·1	2·1
F_1	Dubec Nikitskii 44 × American 572	163	2·12	254	7162	+13	+16·4	3·4
F_1	Havana × American 572	156	2·10	233	7124	+25	+14·6	10·4
	American 572	149	2·32	223	5682	..	..	..
F_1	American 572 × Sumatra Petioled	142	2·35	239	7364	+29	+26·9	3·9
F_1	Dubec Nikitskii 44 × Sumatra Petioled	166	2·14	260	11307	+40	+36·1	3·9
	Sumatra Petioled	184	2·28	248	7980	..	..	..

NOTES

INDIGENOUS DRUGS OF INDIA

In the course of the discussion on two applications for research grants for investigations on indigenous drugs, it was brought to notice that information was not conveniently available on Indian drug-producing plants. Col R. N. Chopra, I.M.S., Professor of Pharmacology, School of Tropical Medicine, Calcutta, has kindly furnished the following lists of:—

A. Important indigenous drugs for which there is a good market.

B. Pharmacopoeial drugs or their substitutes, which grow in India.

Further details will be found in Col. Chopra's book "The indigenous drugs of India".

LIST OF IMPORTANT INDIGENOUS DRUGS GROWING OR CULTIVATED IN INDIA FOR WHICH THERE IS A GOOD MARKET

1. *Abroma augusta*—throughout India.
2. *Abrus precatorius*—throughout India.
3. *Acalypha indica*—throughout India.
4. *Achillea millefolium*—Western Himalayas, cultivated in gardens.
5. *Achyranthes aspera*—throughout India.
6. *Adhatoda vasica*—throughout India.
7. *Aegle marmelos*—throughout India.
8. *Aesculus hippocastanum*—in the Punjab.
9. *Agave americana*—throughout India.
10. *Alangium lamarckii*—throughout India.
11. *Alpinia galanga*—E. Bengal and South India.
12. *Alstonia scholaris*—throughout India.
13. *Amomum subulatum*—throughout India.
14. *Anacyclus pyrethrum*—throughout India.
15. *Anamirta cocculus*—Concan, Malabar, E. Bengal and Assam.
16. *Andrachne cordifolia*—Punjab.
17. *Andrographis paniculata*—throughout India.
18. *Andropogan iwarancusa*—cultivated throughout India.
19. *Anona squamosa*—throughout India.
20. *Anthemis nobilis*—Northern India.
21. *Antiaris toxicaria*—Burma.
22. *Argemone mexicana*—throughout India.

23. *Aristolochia bracteata.*
24. *Bassia latifolia*—throughout India.
25. *Berberis aristata, asiatica*—Himalayas, Nilgiris and Ceylon.
26. *Blumea lacera*—throughout India.
27. *Boerhaavia diffusa*—throughout India.
28. *Butea frondosa*—throughout India.
29. *Caesalpinia bonducella*—throughout India.
30. *Calotropis gigantea*—throughout India.
31. *Cedrus libani*—throughout India.
32. *Cuminum cyminum*—throughout India.
33. *Datura stramonium*—Punjab.
34. *Euonymus crenulatus*—Temperate and Western Himalayas.
35. *Euphorbia pillulifera*—throughout India.
36. *Fumaria officinalis*—Chittoor and Punjab.
37. *Gaultheria fragrantissima*—Nilgiris.
38. *Gossypium herbaceum*—throughout India.
39. *Hedyotis auricularia.*
40. *Helleborus niger*—throughout India.
41. *Holarrhena antidysenterica*—throughout India.
42. *Hygrophila spinosa*—throughout India.
43. *Ipomaea hederaceae*—throughout India.
44. *Ipomaea turpethum*—throughout India.
45. *Mallotus philippinensis*—throughout India.
46. *Melia azadirachta*—throughout India.
47. *Ocimum basilicum*—throughout India (*O. gratissinum*).
48. *Oldenlandia biflora*—throughout India.
49. *Oroxylum indicum*—throughout India.
50. *Peganum harmala*—N.-W. India, Sind, Punjab, Kashmir, Agra and Western Deccan.
51. *Petroselinum sativum, P. graveolensis.*
52. *Picrasma quassioides*—subtropical Himalayas and Kashmir.
53. *Piper cubeba, P. betle*—throughout India.
54. *Plumbago rosea*—throughout India.
55. *Pongamia glabra*—throughout India.
56. *Psoralia corylifolia*—throughout India.
57. *Quercus infectoria*—scattered throughout India.

58. *Rauwolfia serpentina*—tropical Himalayas and at the foot of the hills from Moradabad to Sikkim, Assam, Pegu, Travancore and Ceylon.
59. *Saraca indica*—throughout India.
60. *Scopolia lurida*—Central Himalayas, Nepal, Sikkim.
61. *Semicarpus anacardium*—throughout India.
62. *Sida cordifolia*—throughout India.
63. *Solanum dulcamara, S. indicum*—throughout India.
64. *Swertia chirata*—from Kashmir to Bhutan and Khasia range, at 4,000—10,000 feet.
65. *Symplocos racemosa*—throughout India.
66. *Terminalia arjuna*—throughout India.
67. *Tinospora cordifolia*—throughout India.
68. *Toddalia aculeata*—Nilgiris and from Kumaun to Bhutan, upto 5,000 feet.
69. *Tribulus terrestris*—throughout India and Ceylon.
70. *Tylophora asthmatica*—Madras.
71. *Vernonia anthelmintica*—throughout India.
72. *Viola odorata*—Kashmir, Northern India.
73. *Withania somnifera*—dry subtropical India, west coast.

LIST OF PHARMACOPOEIAL DRUGS OR THEIR SUBSTITUTES GROWING IN INDIA

1. *Acacia arabica*—Indian substitute *Acacia indica*—throughout India.
2. *Aconitum napellus*, grows abundantly. *A chasmanthum Stapf.*—In Kashmir, Bhutan and Tibet.
3. *Aloe chinensis* (*A. perry* and other species, specially thyrox)—Southern India. Substitute *A. indica.*
4. *Arachis hypogoea*—throughout India.
5. *Artemisia maritima*—from Kumaun to Kashmir at a height of 4,000 to 12,000 feet. More abundantly in Baluchistan, Chitral, Afghanistan and in the Himalayas.
6. *Astragalus fucumifr*—imported from Persia. Indian substitute *A. heratensis, A. strobiliferus.*
7. *Atropa belladonna*—Western Himalayas.
8. *Balsamodendron myrrha* and other species—Central Provinces, Cutch and Sind.
9. *Brassic nigra, B. campestris* and *B. juncea*—cultivated universally.

10. *Camellia Thea* and *Coffea arabica*—grow in Darjeeling, Assam, Sylhet, Ceylon and the forests of Southern India.
11. *Cannabis sativa* (or *indica*)—throughout India.
12. *Capsicum*—throughout India.
13. *Carum copticum*—throughout India.
14. *Carum carui*—throughout India.
15. *Cassia fistula*—throughout India.
16. *Cassia augustifolia* and *acutifolia*—throughout India.
17. *Chenopodium ambrosoides*, var. *anthelminticum*—Baluchistan.
18. *Cinchona succirubra* and other varieties—Sikkim, Nilgiri hills, Mungpoo and Ceylon.
19. *Cinnamomum camphora*—bulk imported in India.
20. *Citrus aurantium*—throughout India.
21. *Citrullus colocynthis*—North-West, Central and South India.
22. *Colchicum autumnale* and *luteum*—Kashmir and common in Badghis and Khorasan.
23. *Convolvulus scammonia*.
24. *Coriandrum sativum*—throughout India.
25. *Digitalis purpurea*—cultivated in Kashmir.
26. *Dorema ammoniacum* and other species—Baluchistan.
27. *Elettaria cardamomum*—Western and Southern India.
28. *Eugenia caryophylata*—Malabar coast and Ceylon.
29. *Ephedra vulgaris* and allied species—dry stony hills of north-west Afghanistan, Baluchistan, intermediate Himalayas, Kumaon.
30. *Eucalyptus globulus*—Himalayas.
31. *Ferula foetida*, Indian substitute *F. narthex*—Afghanistan.
32. *Foeniculum vulgare*—cultivated throughout India.
33. *Gentiana lutea*, Indian substitute *G. kurroa*—Kashmir, north-west Himalayas.
34. *Glycyrrhiza glabra*—introduced into Punjab and Sind.
35. *Hyoscyamus niger* and *muticus*—Kashmir and north-west Himalayas.
36. *Juniper communis*, *macropoda*—western Himalayas.
37. *Linum usitatissimum*—cultivated throughout India.
38. *Lobelia inflata*, Indian substitute *nicotianifolia*—Bombay to Travancore and Ceylon.
39. *Mentha arvensis* and *piperita*—Western Himalayas, also cultivated in gardens throughout India.

40. *Myristica fragrans*—cultivated in South India.
41. *Papaver somniferum*—Bihar, U. P. and Punjab.
42. *Peucedanum graveolens*—throughout India.
43. *Picroena excelsa*—Kashmir and north-west Himalayas.
44. *Picrorrhiza kurrooa*—Alpine Himalayas from Kashmir to Sikkim.
45. *Pipenella anisum*—cultivated throughout India.
46. *Pinus longifolia*—Himalayas.
47. *Podophyllum emodi*—N.-W. Himalayas.
48. *Polygala senega*, Indian substitute *P. chinensis, crotalariodes*—throughout India.
49. *Psychotria ipecacuanha*—cultivated in Nilgiris, Mungpoo, Burma.
50. *Ricinus communis*—cultivated throughout India.
51. *Rheum emodi*—Himalayas.
52. *Santalum album*—South India.
53. *Strophanthus.*
54. *Strychnos nux vomica*—specially South India, and Cuttack in B. & O.
55. *Taraktogenos kurzii* and *Hydnocarpus wightiana*—Western Peninsula, South Concan to Travancore.
56. *Thymus vulgaris* (*Corum copticum*)—Indore and Nidam's Domisions.
57. *Urginea indica*—throughout India.
58. *Valeriana wallichii* and *officinale*—N.-W. Himalayas.
59. *Zingiber officinale*—cultivated throughout India.

THE MAYNARD-GANGA RAM PRIZE

In 1925 the late Sir Ganga Ram, Kt., C.I.E., M.V.O., R.B., Lahore, with that generosity for which he was so well known, handed over to the Punjab Government a sum of Rs. 25,000 for the endowment of prize of the value of Rs. 3,000 to be called the Maynard-Ganga Ram Prize and to be awarded every three years, for a discovery, or an invention, or a new practical method which will tend to increase agricultural production in the Punjab on a paying basis. The competition is open to all throughout the world. Government servants are also eligible to compete for it.

Entries for the next award were invited by the 31st December, 1933. None of the entries was considered to be of sufficient merit and it has been decided by the Managing Committee of the prize that the award should be postponed for another year and that further entries should reach the Director of Agriculture, Punjab, Lahore, on or before the 31st December, 1935.

Applications are invited for the Maynard-Ganga Ram Prize of the value of Rs. 3,000 which will be awarded for a discovery, or an invention, or a new practical method tending to increase agricultural production in the Punjab on a paying basis. The prize is open to all, irrespective of caste, creed or nationality and Government servants are also eligible for it. Essays and theses are not eligible for competition and applicants should prove that some part of their discovery, invention, etc., is the result of work done after the prize was founded in 1925. The Managing Committee reserves to itself the right of withholding or postponing the prize, if no satisfactory achievement is reported to it. All entries in competition for the next award should reach the Director of Agriculture, Punjab, Lahore, on or before the 31st December, 1935.

THE KATAMORPHISM OF IGNEOUS ROCKS UNDER HUMID TROPICAL CONDITIONS

BY

THE LATE PROFESSOR SIR JOHN BURCHMORE HARRISON, KT., C.M.G., M.A., F.I.C., F.C.S., F.G.S., F.G.S.A., ETC.,

Late Director of Science and Agriculture, Government Analyst and Geologist, British Guiana

(Published by The Imperial Bureau of Soil Science, Rothamsted Experimental Station, Harpenden—Price 5*s*.)

Sir John Burchmore Harrison, late Director of the Department of Science and Agriculture in British Guinea, died in 1926, leaving behind him the unrevised manuscript of what was perhaps his greatest work. Throughout the 37 years he spent in British Guiana he interested himself in the study of the processes of tropical weathering to which he directed his great powers of observation and analysis. He incorporated the results and conclusion of his life's work on the subject in a manuscript which was discovered by Mr. R. R. Follett Smith of the British Guiana Department of Agriculture in 1930, and forwarded to the Soil Bureau by Professor F. Hardy, of the Imperial College of Tropical Agriculture. The Bureau does not undertake the publication of original research work, but in this instance it has been privileged to do so on behalf of Demerara Proprietors, Ltd., and the British Association for the Advancement of Science, who have generously contributed the necessary funds. The laborious and difficult task of revising the manuscript after the death of its author has been carried out by Professor Hardy. The scientific world owes a debt of gratitude to the contributing bodies and to Professor Hardy for securing the publication of a work of unique value which could never have been printed without their assistance. It is doubtful whether such a detailed and far-reaching study of tropical soil-forming processes, in particular of lateritisation, has ever been made before, and (under present-day conditions) it is most unlikely that the opportunity and ability to repeat Harrison's work will again occur together. It may truthfully be said that it scarcely needs repetition, but it will serve as a sure foundation to further researches on a problem of increasing scientific and economic importance. The monograph should become a standard work of reference to all students of tropical geology and soils.

CONTENTS

Part I—Introductory.

Part II—Katamorphism of Basic Igneous Rocks under Humid Tropical Conditions.

Part III—Katamorphism of Intermediate Igneous Rocks under Humid Tropical Conditions.

Part IV—Katamorphism of Acidic Igneous Rocks under Humid Tropical Conditions.

(1) The Katamorphism of Alkali-Felspar Pegmatite at Mazaruni Quarry.
(2) The Katamorphism of Muscovite Mica at Mazaruni.
(3) The Katamorphism of Granite at Kalacoon and at Mazaruni Quarry.

(A) Kalacoon Section,
(B) Mazaruni Quarry Section.

(C) Comparison between the Kalacoon and the Mazaruni Quarry Sections.
(D) The percolating waters at Mazaruni Quarry.

Crown quarto; 80 pp., with a foreword by Sir E. J. Russel and a preface by Professor Hardy. Stiff paper cover, Price 5 Shillings, post free.

APPENDIX

INSTRUCTIONS TO AUTHORS OF PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH*

1. All manuscripts should be clean, clear and carefully revised. Only one side of the paper should be used, and as far as practicable the original type-written copy and not a carbon copy should be sent. Capitals should be sparingly used, and all the necessary punctuations should be done in the MS. and not left for introduction in proofs.

2. The title of a paper should not be lengthy.

3. It is desirable that the MS. should have suitable heads and sub-heads. In numbering the principal divisions of a paper roman numerals should be used. The use of arabic figures and (*a*), (*b*), (*c*), etc., is generally reserved for numbering the sub-divisions coming under each head.

4. Articles submitted for publication either in the *Indian Journal of Agricultural Science* or in the *Indian Journal of Veterinary Science and Animal Husbandry* should be accompanied by abstracts for publication in *Agriculture and Live-stock in India*. Abstracts should be concise, but should be long enough to explain the matter dealt with; ordinarily no abstract should exceed 200 words.

5. When a word or line is intended to be printed in *italics* it should be underlined with a single line, in SM. CAP. with two lines, in CAPITALS with three lines, and when in **Antique** (heavy type) with a wavy line (~~~~~~~~~~).

6. In descriptive matter, numbers under 100 and all numbers occurring at the beginning of a sentence should be in words.

7. Local names for crops, technical operations, etc., should be defined where they first occur in the text, *e.g.*, *rabi* (spring crop). The use of local weights and measures should be avoided as far as possible. Vernacular names, such as *jowar*, *bajri*, should be in italics without a capital letter, and each such name where it first occurs should be followed by its scientific equivalent in brackets, *e.g.*, *jowar* (*Andropogon Sorghum*). It is usual to write the initial letters of varietal names in capitals, *e.g.*, Striped Mauritius, Dharwar-American cotton and Broach cotton.

8. Botanical and zoological names are printed in italics and should be underlined in the MS., *e.g.*, *Triticum vulgare* L.; *Diplodia Corchori* Syd.; *Pyrilla aberrans* Kirby. The International Rules of Botanical Nomenclature and the International Rules of Zoological Nomenclature should be followed. The names of chemical substances should not be written with a capital letter; they are printed in roman type (*e.g.*, calcium carbonate, prussic acid).

* Spare copies of these Instructions can be had on application to the Secretary, Imperial Council of Agricultural Research (Publication Section), New Delhi.

9. The following and similar abbreviations may be used freely:—*viz.*, *e.g.*, *i.e.*, mm. (millimetre), cm. (centimetre), grm. (gramme), mg. (milligramme), c.c. (cubic centimetre), sp. gr. (specific gravity), lb. (pound), cwt. (hundredweight), in. (inch), ft. (foot), oz. (ounce), md. (maund), sr. (seer), ch. (chattack). Other abbreviations should be used sparingly, if at all.

10. References to plates should be given within brackets, without prefixing the word "see" or "cf.", in the MS. itself, and should not be left over for introduction in proofs. For example, "The parasite (Pl. X, fig. 4) was present late in 1906".

11. The word "Table" is preferable to "Statement", and tables should be numbered consecutively in roman figures. Each table should have an explanation as a sub-head. It is more convenient for reference if tables can be printed horizontally; for this purpose they should not exceed in width the printing measure of the page (5 in.). *Example—*

TABLE IV

Results of water-saving experiments on wheat (Pusa 12) at Gungapur, Haripur and Sargodha, 1916-17

Station	No. of irrigations including the preliminary watering	Yield per acre in maunds and seers		Average yield per acre	
		Grain	Straw	Grain	Straw
		Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.
Gungapur	One	12 19½	20 10	9 34	21 17
Haripur	,,	8 31	19 14		
Sargodha	,,	8 12½	25 27½		

12. References to literature, arranged alphabetically according to author's names, should be placed at the end of the article, the various references to each author being arranged chronologically. Each reference should contain the name of the author (with initials), the year of publication, the abbreviated title of the publication, volume and page. In the text the reference should be indicated by the author's name followed by the year of publication enclosed in brackets; when the author's name occurs in the text, the year of publication only need be given in brackets. If reference is made to several articles published by one author in a single year, these should be numbered in sequence and

the number quoted after the year both in the text and in the collected references. This system of referencing is the same as is used in the *Biochemical Journal* with slight modification and will be clear from the following illustration:—

The work of Osborne and Mendel [1919, 1, 2] and Steenbock and Boutwell [1919] had indicated an association of the fat-soluble vitamin with the green parts of plants. This view was examined by Coward and Drummond [1921] who reported that vitamin A was not synthesised by etiolated shoots but that green leaves were active in its formation. Another worker [Wilson, 1922], on the other hand, found that etiolated shoots if given in sufficient quantity could supply the fat-soluble vitamin and that this factor was therefore formed in the absence of light.

REFERENCES

Coward, K. H. and Drummond, J. C. (1921). *Biochem. J.* **15**, 530.
Osborne, T. B. and Mendel, L. B. (1919, 1). *J. Biol. Chem.* **37**, 187.
——————————(1919, 2). *J. Biol. Chem.* **41**, 549.
Steenbock, H. and Boutwell, R. (1919). *J. Biol. Chem.* **41**, 149.
Wilson, J. (1922). *J. Biol. Chem.* **51**, 455.

Abbreviations, as far as possible, should follow the system adopted in "A World List of Scientific Periodicals" published by the Oxford University Press.

13. Papers should be complete when submitted for publication. As alterations and additions at the proof stage cause both additional expense and delay, they should be resorted to as little as possible. In making corrections in proofs the recognized symbols which will be found in the "Standard Dictionary" should be used. Second (page) proofs will be submitted to authors who should return them promptly.

Illustrations

14. As the *format* of the journals has been standardized, the size adopted being crown quarto (about $7\frac{1}{8}$ in. $\times 9\frac{5}{8}$ in. cut), no text-figure, when printed, should exceed $4\frac{1}{2} \times 5$ inches. Figures for plates should be so planned as to fill a crown quarto plate—the maximum space available for figures being $5\frac{3}{4} \times 8$ inches exclusive of that for letterpress printing.

15. Photos or drawings for illustration should accompany the manuscript and each should bear on the reverse side the name of the paper to which it relates together with the title or legend, figure or plate number, and the size to be reproduced. When giving instructions for reduction linear measurements are understood; thus, "half-size" means reduce to half the length and breadth, not half the area. A photograph should not be rolled up, nor pinned, and should always be packed flat. A complete list of plates and figures should always accompany the paper.

16. Line drawings should be made with clear black lines on smooth white paper, preferably Bristol board. Rough paper should be avoided. Care should

be taken that all the lines are drawn firmly; scratchy or grey lines, produced by the ink being thinned down, are not permissible. Drawings should be larger than the required size. All lettering should be neatly and clearly put in, care being taken to make all lettering sufficiently large to stand reduction.

17. For half-tone work, copy should be made on glossy silver paper and of the same size or larger than the size required.

18. For three-colour work, copy may be oil-painting, water-colour, coloured photograph or coloured transparency, and larger than the size required. In preparing copy, one should use only the primary colours, in any combination, as only inks of primary colours are used in printing. Originals can be enlarged, if necessary, but this should be avoided if possible.

19. For detailed instructions regarding preparation of illustrations, it would be of advantage to refer to Mr. C. M. Hutchinson's article on "Photographic illustrations" in the *Agricultural Journal of India*, Vol. XI, Pt. 3, July 1916, and Mr. A. W. Slater's paper on "The Preparation and Reproduction of Scientific Illustrations" in the *Proceedings of the Third Entomological Meeting*, 1919, which has been reprinted as *Bulletin No. 114 of the Agricultural Research Institute, Pusa.*

GIPD—479 IC of AR—30-3-35—850

ORIGINAL ARTICLES

POLYEMBRYONY IN RICE (*ORYZA SATIVA*)*

BY

K. RAMIAH, M.Sc., DIP.AGRI. (CANTAB.), L.AG.,
Paddy Specialist, Coimbatore,

N. PARTHASARATHY, B.A., B.SC.AG.,

AND

S. RAMANUJAM, B.A. (HONS.),
Assistants in Paddy

(Received for publication on 9th October 1934)
(With Plates VIII and IX)

CONTENTS

I. INTRODUCTION

Polyembryony in angiosperms, though not so prevalent as in gymnosperms, is nevertheless of fairly general occurrence and has been known for a long time. The first discovery of this phenomenon is credited to Leeuwenhock, who as early as 1719 found two embryos in orange seeds. Since then, many more examples have been added to this, and to-day the cases on record are numerous. Among Gramineae this phenomenon, is rarer than in other flowering plants, and has been noticed in wheat, oats, rye [Hansen, 1920], *ragi—Eleusine coracana*—[Rangaswami Ayyangar and Krishnaswami, 1930] and sugarcane [Dutt and Subbarao, 1932]. As regards the origin and significance of polyembryony, diverse views are held and these will be briefly considered later, when the origin of this phenomenon in rice is discussed.

* Paper read at the Indian Science Congress, 1934.

II. Occurrence in rice

There are only three reported instances of polyembryony in rice. Komuro [1932] in Japan observed a rice seed, which upon germination produced two plumules. Rodrigo [1925] in Philippines found one seed of a rice variety producing two plumules and two primary radicles, on germination. He later germinated about 107,000 seeds of the variety and got only one seed with two plumules, and considered this phenomenon rare in rice. Jones [1928] in America obtained one seed from a cross between two rice varieties which produced two hybrid seedlings. The two plants were identical and he concluded that they arose from one fertilised egg. Considering the few and isolated instances of this phenomenon, it was believed to be of very rare occurrence in rice. At the Paddy Breeding Station, Coimbatore, however, quite a large number of cases of polyembryony have been observed and studied. Although seeds giving rise to two seedlings (Plate VIII, fig. 1*a*) were more common, some giving rise to triplets (Plate VIII, fig. 1*b*) were also observed.

In the course of examination of the seed beds of F_3s of a cross between two varieties, T. 24 and T. 381, one seed with two seedlings (Plate VIII, fig. 2*a*) each with its coleoptile and primary root was observed in one of the seed beds. Further search for similar cases resulted in the isolation of five more twins, one of them being composed of two lethal albinos (Plate VIII, 2*b*) the latter having been found in a family segregating for albinism. The occurrence of this phenomenon in the hybrid progenies led to the idea of examining the parent varieties for the presence of this character. A large number of seeds in each of the two parent varieties was germinated until the plumule and the radicle had emerged to more than an inch and these were examined individually for polyembryony. It was found that one of the parents, a pure line, T. 24, showed this phenomenon consistently in the proportion of 1 in 1,000 seeds (Plate IX, fig. 1). Out of several cases isolated, the majority was found to be of twos, while a few were triplets. A similar search in the other parent and some other varieties, however, did not reveal this phenomenon. It may be mentioned in this connection, that the regular occurrence of this phenomenon in T. 24 probably has some significance. Some hereditary tendency apparently exists for this particular feature in this strain and this character is exhibited in the hybrid progenies, (F_1s, F_2s and F_3s), wherever this pure line has been one of the parents. It is not to be supposed, however, that this phenomenon does not occur in other cross progenies, as occasionally double embryos were also obtained from some crosses where T. 24 was not involved as a parent.

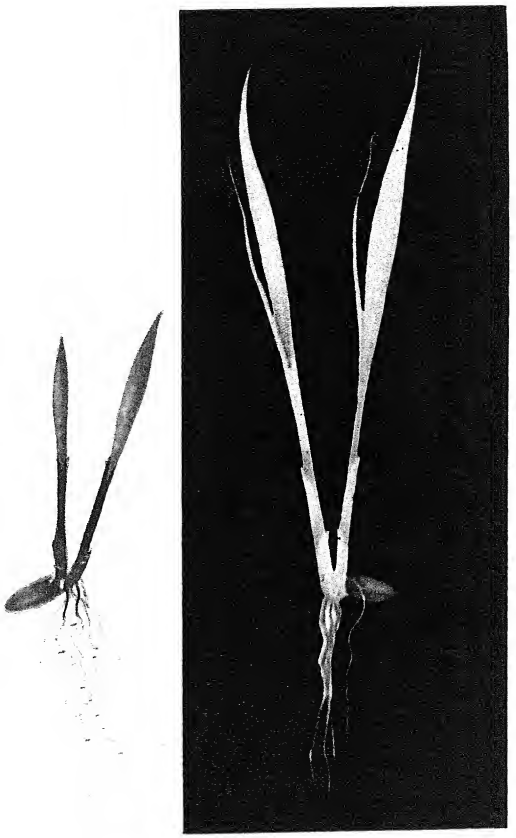
III. Description of cases

The several cases of polyembryony isolated from the pure line, T. 24, and the various hybrid progenies may be classified under two heads :—

(*i*) Genetically identical, and

(*ii*) Genetically different.

FIG. 1.



a *b*

FIG. 2.

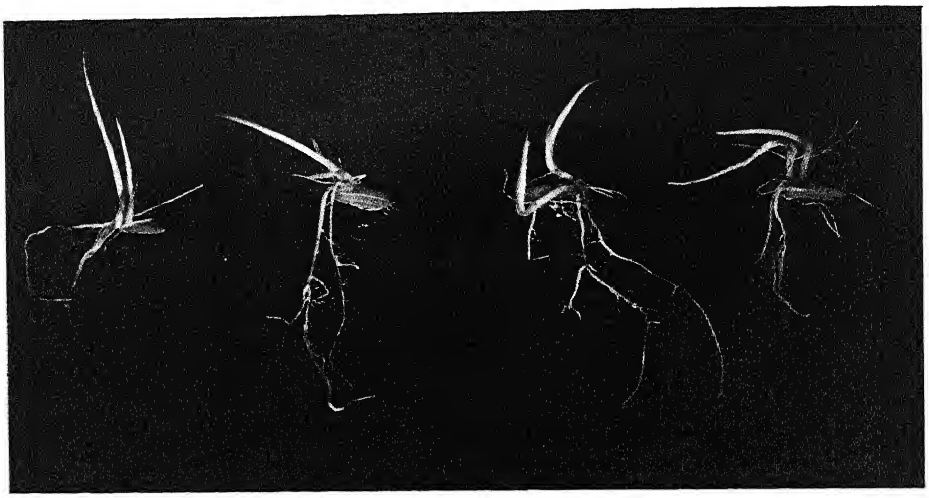


FIG. 1.—Germinated seeds showing polyembryony.

FIG. 2—An albino and a green seedling from a single seed.

1. Genetically identical cases

(*a*) *From the pure line.*—Several twins and a few triplets isolated from the pure line T. 24, were separated and grown to maturity. The individuals constituting the twin or triplet are easily separated after about 10 days' growth in the nursery, when the absorption of the endosperm is complete. All the twins except in one case were found identical in their genetic constitution, vigour of growth, etc., and resembled the parent. Among the triplets, one was always slightly less vigorous than the other two, although all of them were of the same genetic constitution.

(*b*) *From hybrid progenies.*—A number of cases of identical twins were met with in F_1, F_2 and F_3 progenies of crosses between T. 24 and other varieties. Two cases of identical twins were also observed in other hybrid progenies, where T. 24 was not concerned. Among the identical twins, two instances of twin albinos also were met with which, as would be expected, died out early.

2. Genetically different cases

(*a*) *From the pure line.*—By far the most interesting case of polyembryony noted in the pure line is the appearance of a normal diploid individual in association with a haploid from one seed. A description of the haploid is given in a separate publication. [Ramiah, Parthasarathy and Ramanujam ; 1933.]

(*b*) *From hybrid progenies.*—In one of the F_3 families of a cross between two varieties, which was showing a mono-factorial segregation for normal green and albino seedlings, one seed was found to give rise to a normal green and a lethal albino (Plate IX, fig. 2). This association in a single seed is very interesting, as the breeding behaviour of the normal green partner, the albino seedling having died out early, throws some light on the origin of polyembryony in rice. When the green seedling was grown to maturity and the seeds from this plant were sown again, it was found that the progeny consisted entirely of green seedlings, thus showing its homozygous nature with respect to the chlorophyll factor.

IV. Discussion regarding origin and significance

Polyembryony in angiosperms has been observed to arise mostly due to the development of embryos apogamously from cells inside the embryo-sac or from the nucellus and integument by sporophytic budding. Cases where this phenomenon has resulted from the development of distinct embryo-sacs are also recorded and these are classified under pseudo-polyembryony by Ernst [1901] and quoted as such by Coulter and Chamberlain [1903]. The list furnished by Coulter and Chamberlain is fairly exhaustive and shows the diverse sources of origin of this phenomenon in angiosperms. While this feature is rare in certain species, it appears to be the normal method of seed formation in some and is regularly found in certain others, notably in the mango and some of the citrus. Its appearance in several

distinct groups and its origin by several distinct methods from different morphological sources have given rise to diverse views regarding its significance.

As regards the source of polyembryony in rice, the occurrence and breeding behaviour of the green-albino twin, already referred to, throws some light. It suggests that two embryo-sacs of different factorial constitutions are concerned in their development. If we designate **G** as the factor for green colour and **g** for its allelomorph, then the heterozygous plant giving rise to the polyembryonic seed will be **Gg** and the twins themselves **GG**(homozygous green) and **gg**(homozygous albino). It is obvious that the sporophytic tissue of the seed, being itself heterozygous, cannot give rise to homozygous individuals of the constitutions **GG** and **gg** and that the twins should have arisen only from the gametophyte. At gametogenesis, the heterozygous plant would give rise to pollen and embryo-sac mother cells with either the factor **G** or its allelomorph **g** in each. If in this case, as is usual, only one embryo-sac mother cell develops, it can contain only either the factor **G** or its allelomorph **g** and such an embryo-sac cannot give rise to two homozygous individuals of the constitutions **GG** and **gg**. In an embryo-sac containing **G**, the possible embryos that can develop are **GG** and **Gg** by fertilization and **GG** by apogamy. In an embryo-sac with **g**, the possible embryos are **gg** and **Gg** by fertilization and **gg** by apogamy. From the above it is clear that any one embryo-sac cannot give rise to the twins with the constitutions **GG** and **gg** and it is necessary for two embryo-sacs to be formed with different factorial constitutions, for such a phenomenon as a homozygous green and an albino seedlings to develop from the same seed. Such a condition, where more than one embryo-sac has developed in one ovule is not rare in the history of development of the female gametophyte in angiosperms. Coulter and Chamberlain cite a number of examples where more than one embryo-sac has been found to develop. Recently Banerji and Bhaduri [1933] report the presence of two separate embryo-sacs with two well developed embryos in the same ovule of *Nicotiana plumbaginifolia*. Even in rice, a case of two embryo-sac mother cells in one ovule has been observed and figured by Kuwada [1910]. Hence, besides other forms of polyembryony, like nucellar embryony, etc., which may exist in rice, that developing from two embryo-sacs is also a possible source of this phenomenon. Detailed embryological studies are underway to confirm this observation.

Regarding the significance of polyembryony in general, several views are held as already stated. Until a more thorough knowledge of this phenomenon is gained no definite statements on this point would be justified as all such could at best be only suggestions. Strasburger [1877, cited by Tiwary] considered it as a gradual change from the normal formation of the sexually produced embryo, as the result of weakening of sexuality, of pollen formation and of the capacity of the embryo to develop. Pfeffer [1897, cited by Tiwary] has suggested that it is merely a form of budding, the conditions in the embryo-sac giving it the form of the sexually produced embryo. Ganong [1898] on the other hand suggests that it may be the

early stages in the development of some thing new. In its origin in several distinct groups " it is comparable with the appearance of heterospory, but whether polyembryony like heterospory will lead to some higher condition remains to be seen, though we shall not see it." Echinger [1910, cited by Tiwary] believed it to be an attempt on the part of the plant to increase its capacity for dispersal. Ernst [1918, cited by Tiwary] does not subscribe to any of these views but maintains that it is the outcome of the hybrid nature of the plants that exhibit it, and is a form of apospory. He holds that hybridisation is the initial cause of parthenogenesis, apospory, apogamy, nucellar embryony and polyembryony. Chauveaud [1892, cited by Coulter and Chamberlain] considers that polyembryony is a primitive feature of angiosperms, the number having got reduced in the interest of a strong embryo. He found in *Vincetoxiam nigrum* and *V. medium*, polyembryony being a regular feature and also noted four or five bodies in the pollen tube which he thought might be interpreted as male nuclei being responsible for the phenomenon. Dutt and Krishnaswami [1932] have recently found more than two male nuclei in the pollen tube of sugarcane, which may be capable of effecting additional fertilisations and causing polyembryony. Dodel [1891, cited by Coulter and Chamberlain] in describing synergid fertilisation in *Iris siberica* implies somewhat a similar view with that of Chauveaud regarding the significance of polyembryony. Recently Kappert [1933] in an elaborate study of polyembryony in flax consisting of a detailed analysis of different crosses concludes, that polyembryony is a recessive character probably conditioned by a series of multiple allelomorphs. He suggests that in hybridisation, suitable recombination of the recessive multiple factors from various strains occurs, as a consequence of which polyembryony segregates out in certain lines. Our recent observations in the F_4 progenies of a cross between T. 24 and T. 260 have shown several families exhibiting this phenomenon in large numbers. This fact coupled with the occurrence of this phenomenon in T. 24 with a regularity, amounting almost to a hereditary tendency is suggestive of this feature being a heritable character in rice. A detailed analysis of the several cross progenies in which this character is found to segregate is underway and it is expected will throw light on this point.

V. Summary

1. Polyembryony which is believed to be a rare phenomenon in rice, was observed to occur in fairly large numbers at the Paddy Breeding Station, Coimbatore, in a pure line T. 24 and in several hybrid progenies, the pure line alone exhibiting the feature consistently in the proportion of 1 in 1,000 seeds. Although seeds giving rise to two seedlings were more common, some giving rise to triplets, which is being recorded for the first time in rice, were also observed.

2. The several cases of polyembryony isolated were classified and described. Among genetically identical twins, besides the normal green, some lethal albino twins were also observed. Genetically different twins included cases where a haploid was found in association with a diploid and a green seedling with an albino.

3. The occurrence and breeding behaviour of some of the twins are discussed in relation to the origin of polyembryony in rice, and it is tentatively suggested subject to confirmation by further embryological studies in progress, that the development of more than one embryo-sac might also contribute to the origin of this phenomenon. As regards its significance, which is being investigated in more detail, evidences are available which show it to be a hereditary character.

REFERENCES

Bannerji, I. and Bhaduri, P. N. (1933). *Current Sci.* **1**, 310-311.

Coulter, Y. M. and Chamberlain, C. J. (1903). "Morphology of Angiosperms", New York.

Dutt, N. L. and Krishnaswami, M. K. (1932). *Ind. J. Agric. Sci.* **2**, 47-49.

Dutt, N. L. and Subbarao, K. S. (1933). *Ind. J. Agric. Sci.* **3**, 37-53.

Ganong, W. F. (1898). *Bot. Gaz.* **25**, 221-228.

Hansen, W. (1921). *Abs. in Expt. Stat. Rec.* **45**, 533.

Jones, J. W. (1928). *J. Amer. Soc. Agron.* **20**, 533.

Kappert, H. (1933). *Plant Breeding Abs.* **4**, No. 1, item 201.

Komuro, H. (1922). *Bot. Mag. Tokyo* **36**, 23-24.

Kuwada, Z. (1910). *Bot. Mag. Tokyo.* **24**, 267-281.

Ramiah, K., Parthasarathy, N. and Ramanujam, S. (1933). *Current Sci.* **1**, 277-278.

Rangaswami Ayyangar, G. N., and Krishnawswami, N. (1930). *Mad. Agric. J.* **18**, 393-95.

Rodrigo, P. (1925). *Philip. Agric.* **14**, 629-630.

Tiwary, N. K. (1926). *J. Ind. Bot. Soc.* **5**, 124-136.

POLLINATION STUDIES IN *TORIA* (*BRASSICA NAPUS* L. VAR. *DICHOTOMA*, PRAIN) AND *SARSON* (*BRASSICA CAMPESTRIS* L. VAR. *SARSON*, PRAIN)*

BY

ALI MOHAMMAD, M.Sc. (AGRI.), L.AG.,

Botanist for Oilseeds, Punjab Agricultural College, Lyallpur

(Received for publication on 18th January 1935)

CONTENTS

* The original paper with a few more details was submitted to the Pubjab University for the degree of M.Sc. (Ag.) and was accepted.

I. Importance of *toria* and *sarson* as oilseed crops in the Punjab

Toria and *sarson* for which the botanical names *Brassica napus* L. var. *dichotoma* Prain, and *Brassica campestris* L. var. *sarson* Prain, have been used in this paper on the authority of Prain [1898], are by far the most important oilseed crops of the Punjab. *Toria* is chiefly grown in the canal colonies and on the average of five years ending 1932-33 occupied an area of 554,892 acres annually of which 487,881 acres were irrigated. *Sarson* is sown almost all over the province except in some of the hills. The area under this crop is not recorded separately in the official records but is given with *taramira* (*Eruca sativa*) and *rai* (*Brassica juncea*). The total area under these three crops based on the average of five years ending 1932-33 was 643,378 acres each year out of which 93,519 acres were irrigated. The fact that *sarson* is generally grown mixed with gram, barley, or wheat, and rarely alone makes the exact determination of the area under this crop rather difficult, but from a rough estimate of its acreage in various districts, supplied by the revenue authorities concerned, it appears that *sarson* constitutes about 30 per cent of the area recorded under these three crops jointly.

Toria seldom succeeds without irrigation and is more liable to suffer from cold and frost. It is, therefore, usually sown earlier than other *Rabi* (winter) oilseed crops and is treated as *zaid Kharif* (autumn crop). It is generally sown in September and is harvested in December-January.

Sarson is usually sown in October-November almost entirely on unirrigated areas and is harvested in February-March. When sown mixed with other crops the seed is dropped in furrows made by a plough at intervals of four or five feet.

Besides extraction of oil from its seed, *sarson* crop is also used largely as fodder and vegetable. *Toria*, on the other hand, is grown almost entirely for the extraction of oil from its seed. Its importance lies chiefly in the fact that its produce is available, for sale, to the farmer at the time when he needs money for paying off land revenue.

II. Importance of studying modes of pollination

From the plant breeder's point of view the methods employed for the improvement of a crop depend, to a large extent, on the mode of pollination of the crop concerned. A study of pollination methods is extremely necessary, as, in light of these studies the right lines of effecting improvement in a crop can be decided upon and adopted, thus avoiding considerable waste of time and labour. The embryo, which gives rise to an adult plant, results from the fusion of two (male and female) gametes or reproductive cells, the constitution of which may be identical or different. In case these two gametes have, habitually, the same constitution that is to say, the plant is "self-fertilized" from generation to generation, "the like will produce like". The main process of bringing about improvement

n such crop plants is, in principle, a simple one. It consists in selecting, from the local material, a large number of plants differing from one another, multiplying their seed, subjecting them to suitable trials, and then choosing from amongst them, for introduction into general cultivation, one or more which turn out to be most suitable for the conditions prevailing in the locality concerned. Even if there be an occasional variation from this mode of pollination, this method is still applicable. On the other hand, if the two gametes fusing together are always different in constitution or frequently so, that is, a large amount of cross-fertilization occurs in the plant, one generation must be different from the other. In such cases the evolution of unit species and keeping them constant from year to year would be extremely difficult and almost impossible in practice, and from the further fact that almost all such unit species are known to be less vigorous than the naturally occurring heterozygous material the evolution of such unit species would not appear, from the economic standpoint, to be of primary importance. In such crops some type of group-breeding would appear to be the more practical and easier method for effecting improvement.

III. Review of past work

So far as the two crops under consideration, *viz.*, *toria* and *sarson*, are concerned little work appears to have been done in studying their modes of pollination. Some excellent work appears to have been done on the fertility of the genus Brassica in Europe. But *toria* and *sarson* are not the commercial crops of Europe and it is also known that in this genus of plants there exists a wide variation in fertility from species to species and even between varieties within one and the same species, therefore, the work done in Europe does not exactly bear on the subject matter contained in this paper. Even in India, where these two crops are commercially important, there is not much reference available pertaining to this subject, except some preliminary investigations conducted by Howard, Howard and Rahman Khan [1910]; they say that "*Sarson* readily sets seed under bag and a certain amount of self-fertilization is therefore to be expected in free flowering plants". In *toria*, they say, setting under bag is not so easy as in *rai* and *taramira*. Work on *toria* and *sarson* in the Punjab was originally started in the year 1910 by Mr. Milne, C.I.E., then Economic Botanist to Government, Punjab. He collected a number of local varieties of these crops for study. The observations made by him and the author of this paper who worked with him for several years since 1913 showed that considerable crossing occurred in the field in these two crops and that it was very difficult to keep the various varieties pure. No detailed investigations as recorded in this paper were, however, then undertaken. Systematic work on these crops was started in 1926 and developed further in 1929 since when the author of this paper took independent charge of the work on oilseed crops in the Province. The results of some of the breeding investigations on these crops were published [1931] by the author jointly with R. D. Singh and Zafar Alam and it

had been shown that in both these crops, *viz.*, *toria* and *sarson*, the floral mechanism is such that it provides few chances for natural selfing. Under bags they are shy seed setters, the production of normal seeds under bag being about 1/46th and 1/27th respectively, of that in free-flowering branches open to the visits of insects. The yellow seeded form of *sarson* was, however, found to be an exception to this, as it gave quite a good setting under bag. The investigations carried out by the author at Lyallpur in recent years have added greatly to the results obtained previously. The present paper is an attempt to record the important findings up-to-date and it is hoped that it will prove to be a useful contribution towards the elucidation of some of the breeding problems connected with these crops.

IV. Observations and results

(*A*) *Toria*

(*a*) *Floral mechanism and pollination.*—Examination of flowers shows that in the bud-stage immature stamens lie below the stigma with their pollen-liberating sides directed towards the style. As the flower opens, the filaments of the four long stamens increase in length and carry the anthers above the stigma. At the same time these anthers turn half-way round so that their dehiscing surfaces are turned away from the stigma even after the flower has opened; but their anthers remain much below the stigmatic surface. The mechanism is, therefore, not greatly in favour of self-pollination. Each flower has four nectaries. Nectar is profusely secreted by each of the two nectaries situated at the bases of the two short stamens. The other two nectaries which are situated at the bases of the long stamens do not appear to be functional.

The flowers usually open between 8-30 to 10-30 a.m. and as the flowers open the anthers also begin to dehisce from the apex downwards. The dehiscence is usually complete by 12 noon. The flower remains fully open for a day and then it gradually begins to wither and on the fourth or fifth day, after opening, the sepals, petals, and stamens are shed. By this time the fertilized ovary also increases in length.

(*b*) *Insects and pollination.*—The full significance of researches in connection with insects as pollinating agents seems to have been realized only in comparatively recent years. Lewis and Vincent [1909], Chittenden [1914], Morris [1921], Hooper [1922-23], Pammel [1923] and Hutson [1925-26] have definitely established the necessity of honey-bees for the successful pollination of fruit trees like apples, pears, peaches and plums, while Fox Wilson [1929] gives a detailed account of the different species of insects concerned in the pollination of hardy fruits. Insect visits to fruit blossoms greatly increase the percentage of fruit setting and thus materially increase the output. Hendrickson [1916], Overholser [1927] and Farrar [1931], therefore, suggest supplementing of wild insect pollinizers by means of hive-bees. The demand for honey-bees to effect cross-fertilization at the blooming period is steadily increasing in America and according to Phillips [1930] will still

further increase. He, therefore, emphasises that "the requirements for colony strength in order to have bees which will give good service in pollination of fruit" should be thoroughly investigated.

Out of the agricultural crops insect visitors of red clover (*Trifolium partense* L.) seem to have been more extensively studied. Williams [1925] has given an admirable historical resume of the work done in this connection from 1876 to 1925 in different parts of the world. He, and more recently Walton [1927] have established that humble-bees (*Bombus sp.*) are the most important for red clover pollination.

Balls and others [1929] have shown that solitary and ground bees are much more important agents in the natural crossing of cotton flowers in Egypt and consequent varietal deterioration than the honey-bees. Treherne [1923] shows that honey-bees are the most important for seed production in vegetable crops.

In India, the problem of studying insect visitors of farm crops lies practically untouched. Burkill [1906, 1, 2, 3; 1907; 1908; 1911] published a series of notes on the pollination of flowers in India and contributed an article on "Insects and flowers" in Indian Insect Life [Lefroy, 1909, pp. 222-223]. In these contributions Burkill records the names of insect visitors of different kinds of flowers.

The study on the insect visitors of *toria* and *sarson* at Lyallpur with particular reference to the part played by them in the pollination of these crops, was taken up by the author in 1929, when representatives of three orders, *viz.*, Hymenoptera, Lepidoptera, and Diptera were noticed to visit *toria* and *sarson* flowers at Lyallpur. The detailed observations were recorded in later years with the help of Mr. Khan A. Rahman, Assistant Professor of Entomology who kindly helped in the collection and identification, etc., of the insects concerned. One hundred and seventeen different species of insects falling under the following 7 orders (given in order of importance) have been recorded visiting *toria* flowers :—

1. Hymenoptera.
2. Diptera.
3. Lepidoptera.
4. Coleoptera.
5. Rhynchota.
6. Thysanoptera.
7. Neuroptera.

Observations made on the relative abundance of the various species of insects and their relative efficiency in affecting pollination of *toria* showed that *Andrena ilerda* (Hymenoptera) is by far the most important and efficient pollinator of *toria*, and that the female is much more active and useful than the male in the pollination work. Next to *Andrena ilerda* in importance are *Apis florea* (Hymenoptera) and *Halictus sp.* (Hymenoptera). The rest of the insects do not appear to be so useful either because they are met with only in small numbers or because they are indifferent workers; while a few are predators, *e.g.*, *Philanthus depraedator* Smith

and *Liris haemorrhoidatis* F. (Hymenoptera), and therefore, they do more harm than good by killing the useful pollinating insects. Considered from all points of view, *Andrena ilerda*, *Apis florea* and *Halictus sp.* may be regarded as the chief pollinating agents of *toria* as these insects abound in numbers during the main flowering season of *toria* and furthermore are very ardent and persistent workers. Between them these three insects have been recorded to make up on the average about 82 per cent of the insect visitors to *toria* flowers as follows :—*Andrena ilerda* 54 per cent, *Apis florea* 19 per cent, *Halictus sp.* 9 per cent.

As already pointed out above, the floral mechanism in *toria* is such as to reduce the chances for self-pollination to the minimum. Therefore, the flowers must depend on some external agency for bringing about pollination. Observations made in this connection show that insects play a great part in the pollination and that wind hardly plays any role, if at all, in this respect. The insects start visiting the flowers at 10 a.m. and are met with in largest numbers between 12 noon and 2 p.m. After 3 p.m. their number decreases considerably, so much so that after 4 p.m. hardly any insect can be seen visiting the flowers. Insects visit only freshly opened flowers for the collection of the nectar, which is secreted by the nectaries only on the day the flower opens. It is interesting to note that an examination of the anthers of freshly opened flowers at about 3 p.m. shows them to be absolutely devoid of their pollen which is evidently carried away by insect visitors.

Detailed observations were recorded during the year 1929-30 with regard to the frequency of insect visits to flowers. For this purpose, a number of individual flowers and inflorescences were earmarked at different places in the field and the number of insects visiting them per hour was recorded for about a fortnight from 9 a.m. to 5 p.m. daily. The results show that, on the average, an inflorescence and a flower were visited by insects 71 and 26 times respectively per day. The frequency of insect visits is so great that there is absolutely no chance for a flower to escape pollination through the agency of insects. It may, however, be pointed out that the activity of insects is largely controlled by weather. It has been ascertained that very few insects come out to visit flowers on cloudy days and the few that do come out remain lethargic. A comparison of the number of hourly visits by insects to an individual inflorescence on a cloudy and a clear day is given below :—

Comparison of frequency of insect visits on a clear and a cloudy day

Kind of weather	Number of insect visitors between								
	9 & 10 a.m.	10 & 11 a.m.	11 & 12 a.m.	12 a.m. & 1 p.m.	1 & 2 p.m.	2 & 3 p.m.	3 & 4 p.m.	4 & 5 p.m.	Total
Clear day . .	..	13	20	29	42	28	21	..	153
Cloudy day . .	..	..	1	..	..	2	..	..	3

It is apparent from the above that the number of insects visiting the flowers on a clear day is far greater than those visiting the flowers on a cloudy day Naturally, therefore, whatever setting occurs on cloudy days takes place from natural self-pollination, the extent of which in this crop, as will be shown later, is very little. From these facts it follows that the intervention of long spells of cloudy, humid, or rainy weather during the flowering period of *toria* would decrease the percentage of setting. It has actually been observed that the flowers opening on a cloudy, humid day do not set into pods, with the result that, if such weather lasts sufficiently long, considerable podless gaps appear on the fruiting branches.

It may also be noted that, unlike cotton pollen, *toria*, and *sarson* pollen grains do not burst on coming into contact with free water and that the flowers crossed artificially on a cloudy day set into normal pods. Therefore, the only possible explanation is that the reduction in setting with the intervention of cloudy, humid and rainy weather is simply the result of a reduction in the visits of pollinating insects.

(*B*) *Sarson*

Floral mechanism and mode of pollination.—The floral mechanism in all brown seeded forms of *sarson*, that have been under observation so far, is the same as in *oria*, and hence the chances for natural self-fertilization are few. But one form of *sarson*, which is yellow-seeded and is grown to a limited extent in some parts of the North Western district of the Province, has been found to differ in this respect. In this case, the pollen-liberating sides of the anthers of the long stamens remain towards the stigma even in a fully opened flower. These anthers at the time of dehiscence meet over the stigma and then begin to turn away from the stigmatic surface, each forming a sort of coil. A little before, or during this process, some pollen drops on the stigma so that a good amount of self-fertilization can be expected. The anthers of the short stamens do not, however, reach the stigma and, therefore, do not appear to take any part in the process of self-pollination. As reagrds the mode of pollination, varieties of all *sarson* with the exception of this particular form, have been found to behave in the same manner as *toria*. Generally speaking, the same species of insects which visit *toria* flowers have been found to visit *sarson* flowers also with the only difference that their relative abundance in the case of *sarson* crop is not usually the same as that found in the case of *toria* crop. For example, in *toria* crop, *Andrena ilerda* occupied the premier place, but in the case of *sarson* where this insect is by no means an inappreciable agent in pollination work, *Apis florea* is met with in such large numbers and is such a persistent worker that it should be regarded as the most important pollinating agent of *sarson*. The main flowering season of *sarson* crop usually comes about $1\frac{1}{2}$ to 2 months later than that of *toria* and it is very likely that during this interval *Apis florea* finds more favourable conditions and consequently its population increases. Further researches in this direction are being conducted in collaboration with the Entomological Section.

(C) *Self and cross-fertilization in* toria *and* sarson

In order to explore the possibilities of the isolation of pure lines in *toria* and *sarson*, attempts were made to secure their self-fertilized seed by enclosing plants in muslin bags. It was repeatedly observed that in these two crops, except in the yellow seeded form already referred to above, little setting resulted, and consequently a sufficient supply of seed from a single plant could not be obtained. Percentage setting under bag was determined in each of the years 1926-27 to 1931-32 (Table I).

TABLE I

Result of setting under bag in toria *and* sarson *for the years 1926-27 to 1931-32*

Year	Toria			Sarson					
				Brown seeded			Yellow seeded		
	No. of flowers bagged	No. of pods set	Percentage of setting	No. of flowers bagged	No. of pods set	Percentage of setting	No. of flowers bagged	No. of pods set	Percentage of setting
1926-27 . .	3,328	282	8·4	10,855	1,773	16·3	38	38	100
1927-28 . .	7,357	1,147	15·5	606	185	30·5	162	135	83·3
1928-29 . .	8,052	677	8·4	1,137	263	23·1	...	...	...
1929-30 . .	1,019	277	27·1	3,614	1,047	28·9	...	...	...
1930-31 . .	2,201	203	9·2	2,714	595	22·3	45	44	97·8
1931-32 . .	1,238	266	21·4	3,200	628	19·6	189	178	94·2
Total or average	23,195	2,852	12·3	22,126	4,491	20·3	434	395	91·0

From these figures it will be seen that on the average of six years there is a pod-setting under bag 12·3 per cent and 20·3 per cent respectively in *toria* and brown-seeded *sarson*. It may be pointed out, however, that the actual seed formation is very much less than what the figures of pod setting show because the pods, which succeed in setting inside bags, contain very much fewer seeds than the pods formed on free-flowering plants.

With a view to determine whether the very poor setting obtained under bag was entirely due to the floral mechanism providing few chances for pollination or to some other causes also, and also to find out if with the aid of artificial self-pollination self-fertilized seed from individual plants could be had in quantities sufficient to permit the isolation of pure lines, certain experiments were carried out on a number of *toria* plants in the year 1927-28. For this purpose many plants were

selected and different branches of the same plant were accorded one of the following three treatments :—

(*a*) One branch was left free-flowering, *i.e.*, open to the visits of insects.

(*b*) One branch was bagged but otherwise left untouched, and

(*c*) One branch was bagged but freshly opened flowers were each day self-pollinated by means of a camel hair brush. The results so obtained (Table II) show that on the average, for every 100 flowers treated, artificial selfing by means of hand-pollination increased the amount of both pod-setting and seed production from 14·5 and 27 to 33·6 and 90 respectively. But these figures are far less than those obtained from free-flowering branches, in which case 60 pods and 1,086 seeds were formed per 100 flowers. It will also be seen that although artificial selfing appreciably increased the pod-setting, yet there was very little increase in the seed production per pod, as compared to those obtained in free-flowering branches. This shows that pod-formation and seed-formation in *toria* were, in these experiments, largely independent of each other. This is in accordance with the results obtained by Nelson [1927] in some selfing experiments with some other forms of the genus *Brassica*.

TABLE II

Comparison of setting in free-flowering, artificially selfed, and naturally selfed flowers in toria *in the year 1927-28*

Plant No.	Bagged						Bagged but artificially selfed (Hand-pollinated)						Free-flowering					
	Total No. of flowers under observation	Total No. of pods formed	Percentage of pod-setting	Average No. of normal seeds per pod	Total number of normal seeds	Average number of normal seeds calculated per 100 flowers treated	Total number of flowers treated	Total number of pods formed	Percentage of pod-setting	Average number of normal seeds per pod	Total number of normal seeds	Average number of normal seeds calculated per 100 flowers	Total number of flowers under observation	Total number of pods formed	Percentage of pod-setting	Average number of normal seeds per pod	Total number of normal seeds	Number of normal seeds formed per 100 flowers
1 . .	289	123	42·6	2·62	322	...	41	37	90·2	4·36	161	...	62	50	80·6	22·18	1,109	...
2 . .	679	93	13·7	1·40	130	...	51	36	70·6	2·58	93	...	102	63	61·8	17·90	1,127	...
3 . .	151	23	15·2	1·05	24	...	42	20	47·6	1·10	22	...	42	25	59·5	16·52	413	...
4 . .	222	1	0·4	0·00	0	...	86	6	7·0	0·50	3	...	44	20	45·0	10·15	203	...
5 . .	253	16	6·3	1·37	22	...	32	5	15·6	1·20	6	...	57	40	70·2	11·02	441	...
6 . .	266	24	9·0	1·14	27	...	42	2	4·8	1·00	2	...	85	70	82·3	18·23	1,276	...
7 . .	137	10	7·3	1·70	17	27	30	3	10·0	1·66	5	90	80	45	56·2	12·33	555	1,086
Average .	...	...	14·5	...	...	27	...	...	33·6	...	...	90	...	...	66·0	...	...	1,086

In these experiments some damage due to handling or some other cause occurred to flowers, and such damaged flowers have been included in the class of failures. Even if these damaged flowers are excluded from the above results, the average number of normal seeds per 100 flowers in the case of the different treatments would be as follows :—

Treatment	No. of seeds per 100 flowers
Bagged flowers	40
Artificially selfed flowers	186
Unbagged flowers	1,269

These figures, though slightly different, are of the same order as the previous ones and lead to the conclusion that in freshly opened flowers of *toria* even self-pollination by hand does not produce an appreciable amount of seed.

In order to see whether the poor setting under bag, with or without artificial selfing, was in some way due to the effect of bags used for enclosing the branches, experiments were arranged to compare the following three treatments :—

(*a*) Flowers were bagged only.

(*b*) Flowers (freshly opened) under bag were artificially selfed by hand.

(*c*) Flowers (freshly opened) under bag were crossed with pollen from other *toria* plants.

In each set of these experiments the three different treatments were given to three different flowering branches on each plant and the muslin bags used were in all cases of exactly the same type and material. The results obtained (Table III) show that artificially crossed flowers under bag give an excellent setting, immensely greater than in the cases where setting depended on selfing alone, natural or artificial. It will again be seen that while artificial selfing appreciably increased pod-setting, it made no increase in the production per pod of normal seeds. Crossing under bag gives, not only, an immense improvement in setting, but the pods formed are also much bigger and better developed (Table V). From Table V it will be seen that ' selfed ' pods have about half the length of ' crossed ' pods and that the loss in length which results from selfing is mainly confined to the seed-bearing portion of the pod.

TABLE III

Comparison of setting in toria *under bag in untouched, artificially selfed, and crossed flowers in the year 1929-30*

Plant No.	Untouched						Artificially selfed (Hand-pollinated)						Crossed					
	Total number of flowers under observation	Total number of pods formed	Percentage of pod setting	Average number of normal seeds per pod	Total number of normal seeds	Number of normal seeds formed per 100 flowers	Total number of flowers treated	Total number of pods formed	Percentage of pod setting	Average number of normal seeds per pod	Total number of normal seeds	Average number of normal seeds calculated per 100 flowers	Total number of flowers treated	Total number of pods formed	Percentage of pod setting	Average number of normal seeds per pod	Total number of normal seeds	Average number of normal seeds calculated per 100 flowers
1 . .	85	47	55·3	2·3	107	...	68	54	79·4	2·4	131	...	50	50	100·0	22·3	1,114	...
2 . .	45	11	24·4	1·0	11	...	72	29	40·3	3·1	63	...	30	30	100·0	17·8	534	...
3 . .	52	28	53·8	1·3	36	...	80	41	51·2	1·1	45	...	40	40	100·0	14·3	572	...
4 . .	66	7	10·6	1·4	10	66	22	13	59·1	1·4	18	106	20	20	100·0	19·4	388	1,863
Average .	...	...	37·5	...	...	66	...	...	56·6	...	...	106	...	...	100·0	...	...	1,863

Similar experiments conducted on *sarson* in 1929-30 (Table IV) show that the results in this case are also similar to those obtained in the case of *toria*. It will also be seen that even slightly better pod-setting and seed-production were obtained with artificial crossing than in the case of free-flowering branches, which is plausible on the assumption that in the case of free-flowering branches a small number of flowers might have been pollinated with their own pollen.

TABLE IV

Comparison of setting in sarson, *of untouched, artificially selfed and crossed flowers under bag and flowers left unbagged, in the year 1929-30*

Plant No.	Bagged only (otherwise) untouched						Bagged and artificially selfed (Hand-pollinated)						Free flowering						Bagged and crossed					
	Total number of flowers treated	Total number of pods formed	Percentage of setting	Average number of normal seeds per pod	Total number of normal seeds	Number of normal seeds calculated per 100 flowers	Total number of flowers treated	Total number of pods formed	Percentage of pod-setting	Average number of normal seeds per pod	Total number of normal seeds	Average number of normal seeds calculated per 100 flowers	Total number of flowers treated	Total number of pods formed	Percentage of pod-setting	Average number of normal seeds per pod	Total number of normal seeds	Average number of normal seeds calculated per 100 flowers	Total number of flowers treated	Total number of pods formed	Percentage of pod-setting	Average number of normal seeds per pod	Total number of normal seeds	Average number of normal seeds calculated per 100 flowers
1 . .	25	8	32·0	1·4	11	...	25	10	40·0	2·9	29	...	22	18	81·8	14·8	266	...	27	27	100·0	14·2	384	...
2 . .	Branches damaged												10	10	100·0	11·6	116	...	11	11	100·0	13·3	146	...
3 . .	80	17	21·2	1·5	25	...	8	3	37·5	1·3	4	...	20	19	95·0	15·8	300	...	25	25	100·0	20·8	521	...
4 . .	37	7	18·9	2·8	20	...	26	10	38·6	2·8	28	...	16	15	93·7	21·3	319	...	26	26	100·0	19·4	504	...
5 . .	84	33	39·3	2·4	80	60	30	19	63·3	3·7	44	118	10	10	100·0	24·4	244	1,596	17	17	100·0	24·3	411	1,855
Average .	...	...	29	...	...	60	...	...	47	...	...	118	...	...	92	...	...	1,596	...	...	100·0	...	...	1,855

In brown-seeded *sarson* also, as in *toria*, pods formed as a result of crossing are much bigger and contain incomparably greater number of seeds than the pods produced by natural or artificial selfing. Results of experiments bearing on these points, both for *toria* and *sarson*, are tabulated in Table V.

TABLE V

Reduction which takes place on selfing, in toria *and* sarson *in the length of pods and number of normal seeds*

Crop	Year	Treatment of flowering branches	Number of flowers treated	Average length in centimeters per pod				Average No. of normal seeds per pod
				Entire pod	Pedicel	Seed bearing portion	Beak	
Toria . .	1927-28	Bagged . . .	1,997	4·46	1·42	2·05	0·99	1·33
		Free-flowering .	472	6·84	1·53	3·95	1·36	15·48
	1929-30	Bagged . . .	248	4·77	1·67	2·17	0·93	1·50
		Hand-selfed . .	242	5·15	1·72	2·40	1·03	2·00
		Artificially crossed .	140	8·95	1·77	5·42	1·76	18·45
Sarson brown-seeded	1927-28	Bagged . . .	606	5·30	1·50	2·40	1·40	1·86
		Free-flowering .	145	6·60	1·60	3·60	1·40	6·18
	1929-30	Bagged . . .	226	4·72	1·75	2·25	0·72	2·02
		Hand-selfed . .	89	5·02	1·60	2·47	0·93	2·67
		Free-flowering .	68	7·70	1·95	4·35	1·40	19·07
		Artificially . .	95	8·10	1·75	4·90	1·45	19·67
Sarson yellow-seeded	1927-28	Bagged . . .	104	7·10	1·50	4·10	1·50	14·01
		Free-flowering .	91	7·10	1·60	4·30	1·20	17·11

These figures show that in by far the most of the cases natural or artificial selfing in *toria* and brown-seeded *sarson* reduces the length of the pods to $\frac{1}{2}$ to $\frac{2}{3}$ of that given by 'crossed' flowers and the number of seeds per pod to 1/12 to 1/6. As already stated, the loss of length which takes place in the pod is mainly confined to its seed-bearing portion, affects the beak very slightly, and has practically no effect on the pedicel.

From Table V it will also be seen that very little reduction of this sort takes place in the yellow-seeded form of *sarson*. As already shown in Table I above, this particular form of *sarson*, unlike brown-seeded forms, gives an exceptionally good setting under bag due to the floral mechanism affording facilities for self-pollination. As shown in the case of *toria* and brown-seeded forms of *sarson*, facilities for self-pollination alone are not sufficient. The plant must have the quality of self-compatibility, which quality the yellow-seeded form of *sarson* seems actually to possess.

(*D*) *Effect of self-pollination treatment at different stages of flower development on pod and seed setting*

As already stated the different pollination treatments in the experiments referred to above were in each case given to freshly opened flowers, *i.e.*, on the same morning on which the flowers opened. Experiments were carried out both in the case of *toria* and brown-seeded *sarson* in the years 1931-32 and 1932-33 to find out the effect of self-pollinating the stigmas artificially in the flower buds and in the open flowers of various ages. For pollination in the bud stage a number of flower-buds in an inflorescence were self-pollinated on a particular day, and later on the number of flowers was counted as they opened each day. The opened flowers were labelled daily in order to find out the time the bud was self-pollinated, before it opened into a flower. Thus it was possible to trace out the number of days a particular flower was self-pollinated in the bud-stage before opening. In the cases where flowers were self-pollinated certain days after opening, similar records were kept about the days on which flowers opened and these flowers were later self-pollinated at the required time. The results are tabulated in Table VI.

TABLE VI

Results of differential self-pollination treatment on pod and seed-setting in toria *and* sarson *for the years 1931-32 and 1932-33*

Treatment	*Toria*										*Sarson* (Brown-seeded)									
	Total number of flowers treated	Total number of pods formed	Percentage of pod-setting	Average length in cm. per pod				Average number of normal seeds per pod	Total number of normal seeds.	Average number of normal seeds calculated per 100 flowers.	Total number of flowers treated	Total number of pods formed	Percentage of pod-setting	Average length in cm. per pod				Average number of normal seeds per pod	Total number of normal seeds	Average number of normal seeds calculated per 100 flowers
				Entire pod	Pedicel	Seed bearing portion	Beak							Entire pod	Pedicel	Seed-bearing portion	Beak			
Selfed one day before opening	78	40	51·3	6·2	1·9	3·0	1·3	3·4	136	174	80	15	18·8	5·3	1·8	2·0	1·5	1·0	15	19
Selfed two days before opening	76	74	97·4	8·5	1·8	4·8	1·9	9·3	688	905	90	60	66·7	5·9	1·7	2·4	1·8	2·8	168	187
Selfed three days before opening	88	88	100·0	8·7	1·7	4·9	2·1	9·1	801	910	78	78	100·0	8·8	1·8	4.3	2·7	10·2	796	1,021
Selfed four days before opening	76	72	94·7	7·3	1·7	3·3	2·3	5·4	389	512				Damaged						
Selfed same day . . .	145	68	47·6	5·1	1·7	2·3	1·1	1·9	129	89	114	23	20·2	5·0	1·7	1·9	1·4	1·5	35	31
Free flowering . . .	80	78	97·5	7·8	1·7	4·7	1·4	17·4	1,357	1,696	93	90	96·8	8·5	2·0	4·4	2·1	16·4	1,476	1,587
Selfed one day after opening.	71	21	29·6	4·7	1·6	2·1	1·0	2·4	50	70	74	34	45·9	6·1	2·1	2·9	1·1	3·0	102	138
Selfed two days after opening	121	78	64·5	6·1	2·2	2·6	1·3	2·8	218	180	74	51	68·9	6·9	2·1	3·1	1·7	3·0	153	207
Selfed three days after opening	81	60	74·1	5·8	2·1	2·7	1·0	3·4	204	252	89	54	60·7	6·3	1·9	2·7	1·7	3·1	167	188
Selfed four days after opening	84	42	50·0	5·6	2·0	2·5	1·1	2·0	84	100	90	50	55·6	5·1	1·8	2·2	1·1	0·9	45	50
Selfed five days after opening.	78	27	34·6	5·2	2·2	2·2	0·8	1·4	38	49	53	8	15·1	5·8	2·1	2·4	1·3	1·6	13	25
Selfed same day . . .	127	46	36·2	5·2	1·9	2·3	1·0	2·0	92	72	94	42	44·7	5·7	2·0	2·4	1·3	1·0	42	45
Free-flowering . . .	139	135	97·1	8·0	2·1	4·3	1·6	18·0	2,430	1,748	116	111	95·7	7·7	1·9	4·4	1·4	15·9	1,765	1,522

The figures show that by self-pollinating a flower bud two and three days in the case of *toria* and three days in the case of brown-seeded *sarson* before it opens out into a flower, quite a good pod-setting and seed-production is obtained, which compares favourably with that on free flowering branches. In fact by self-pollinating a bud three days before opening cent per cent pod setting has been obtained as compared to 97·5 per cent and 96·8 per cent obtained in the case of free flowering branches in *toria* and *sarson* respectively, so that normally self-incompatible plants become highly self-fertile when flowers are pollinated in bud stage. Similar phenomenon has been reported in the case of some other species of Cruciferae by Kakizaki and Kasai [1933], who designate this phenomenon as 'pseudo-fertility'. It may be argued that the increase in self-fertility by bud-pollination is due to the comparatively smaller length of the styles in the flower-buds than that in the case of opened flowers and, therefore, pollen tubes, which are believed to attain an insufficient length when selfing is resorted to, are not capable of reaching the ovary and fertilizing the ovules. But when further it is considered that in the above experiments the flowers self-pollinated two or three days after the opening gave much better pod-setting and seed production than those selfed on the same day when they opened or one day after their opening, this assumption does not appear to hold good absolutely as the styles are decidedly longer in the two or three days old flowers than in the freshly opened or one day old flowers. Akhtar [1932] working on some Indian Brassicae has concluded that pollen tubes grow more quickly in compatible unions than in incompatible matings and that sterility in incompatible matings is due to the failures of the pollen tubes to reach the ovules before it has lost its receptive power. It has been shown by the investigation of Jost [1907], Martin [1913] and Silow [1931], that self-sterility in red clover (*Trifolium pratense* L.), is due to the slower growth of 'self' pollen than of foreign pollen in the styler tissue and that this may be accounted for by an inhibiting action of that tissue. From the results quoted above, however, it is evident that in the case of *toria* and brown-seeded *sarson* this kind of inibiting action in the styler tissue, is not equally effective in flowers, of different ages. In fact it does not seem to act effectively at all when selfing is done in buds three days before they open into flowers. It, therefore, appears more likely that in the case of *toria* flowers this secretion is produced most actively from one day before opening to one day after opening, while in *sarson* flowers it is produced from about two days before opening to one day after opening. As will be shown later, the fall in the pod-setting and seed production after the flower is more than three days old is due to the fall in the receptivity of the stigma.

(*E*) *Duration of the period of receptivity of stigma and viability of pollen grains*

A detailed study of the period for which the stigma remains receptive and the pollen grains viable was carried out in the years 1930-31 and 1931-32. The

following three different sets of artificial cross-pollination experiments in *toria* and brown-seeded *sarson* were arranged :—

(*a*) Pollen from freshly opened flowers was applied to stigmas of different ages, *viz.*, one to six days after the opening of flowers.

(*b*) Stigmas of freshly opened flowers were treated with pollen grains of varying ages, *viz.*, one to nine days after the opening of flowers.

(*c*) Different combinations of pollen grains and stigmas of varying ages ranging from one to three days after the opening of flowers were tried.

The results obtained by giving differential pollination treatment in each set of these experiments were compared with the results obtained by applying fresh pollen to fresh stigmas and also with those obtained from the free flowering branches of the same plant left open to the visits of insects. From the data obtained (Table VII) the following conclusions are drawn.

(*i*) *Length of the period of receptivity of stigmas as shown by their being treated with fresh pollen.*—Although there was a decline in pod-setting with an advance in the age of the stigma, yet a fair amount of setting was obtained in the case of even four days old stigma. The percentage of pod-setting is, however, very low in the cases of five or six days old stigma. The pod-setting and seed production was normal in the cases where one to two days old stigmas were pollinated. In the case of stigmas, three days old, a decline in the seed production occurred and the number of seeds produced per pod in this case was about 3/5 and 2/3 respectively of those obtained from the pods formed as a result of the application of fresh pollen to fresh stigmas and from the pods formed on free flowering branches. Further, with four days old stigmas a great fall in the number of seeds produced per pod occurred and this fall was still greater in the case of the five and six days old stigmas, the number of seeds per pod in the latter case being only 2·3 in *toria* and 2·7 in brown-seeded *sarson* as against 16·1 and 19·7 respectively obtained from the free flowering branches of these crops. These results therefore, indicate that after the opening of the flower the stigma remains fairly receptive for three days after which time there occurs an appreciable fall in pod and seed production, so much so, that with five days old stigmas very little pod setting and seed formation are obtained. Sepals and petals of *toria* flowers are shed on the fourth or fifth day after the opening of the flowers. Therefore, the decrease in pod setting and seed production seems to be associated with the withering away of sepals and petals so that the condition of sepals and petals in *toria* flowers may serve as an indication of the degree of receptivity of the stigma.

(*ii*) *Length of the period of viability of pollen grains.*—Quite normal pod setting and seed production have been obtained by treating fresh stigmas with pollen grains of varying ages. From the results obtained it is evident that in the case of *toria* sufficient number of pollen grains, to bring about normal setting and seed production, remain viable for seven days after collection from the freshly opened flowers. In

the case of brown-seeded *sarson*, however, they appear to remain viable for even a slightly longer period.

(*iii*) *Stigmas and pollen grains of varying ages*.—Various combinations of one to three days old pollen grains and stigmas have shown quite normal pod setting in almost all cases.

Considering the above results as a whole it is concluded that stigmas in *toria* and *sarson* remain fairly receptive for at least three days after the opening of flowers and pollen grains remain viable for at least seven days. These results have proved very helpful in some of the breeding investigations being carried out on these crops.

TABLE VII

Showing the result of pollination experiments in toria *and* sarson *with stigmas and pollen grains of different ages during the years 1930-31 and 1931-32*

Treatment	TORIA										SARSON (Brown-seeded)									
	Total number of flowers treated	Total number of pods treated	Percentage of pod-setting	Average length in cm. per pod				Average number of normal seeds per pod	Total No. of normal seeds	Average No. of normal seeds calculated per 100 flowers	Total number of flowers treated	Total No. of pods formed	Percentage of pod-setting	Average length in cm. per pod				Average No. of normal seeds per pod	Total No. of normal seeds	Average No. of normal seeds calculated per 100 flowers
				Entire pod	Pedicel	Seed-bearing portion	Beak							Entire pod	Pedicel	Seed-bearing portion	Beak			
One day old stigma and fresh pollen	110	108	98·2	8·8	2·2	5·8	1·0	15·4	1,663	1,512	85	78	91·8	11·2	2·7	6·0	2·5	19·8	1,544	1,816
Two days old stigma and fresh pollen	102	98	96·1	8·5	1·8	5·0	1·7	17·1	1,676	1,643	96	91	94·8	10·0	2·4	5·3	2·3	17·0	1,547	1,611
Three days old stigma and fresh pollen	104	99	95·2	7·6	2·1	4·1	1·4	15·5	1,535	1,476	85	72	84·7	8·6	2·1	4·9	1·6	13·6	97	1,152
Four days old stigma and fresh pollen	100	81	81·0	7·1	2·3	3·0	1·8	4·4	356	356	87	57	65·5	7·2	2·0	3·7	1·5	9·9	564	648
Five days old stigma and fresh pollen	80	34	42·5	5·1	2·1	2·1	0·9	2·3	78	98	105	66	62·9	6·7	1·9	3·5	1·3	7·6	502	478
Six days old stigma and fresh pollen	72	30	41·7	5·1	2·0	2·0	1·1	2·3	69	96	25	4	16·0	6·4	2·2	2·1	2·1	2·7	11	44
Fresh stigma and fresh pollen .	106	104	98·1	9·9	2·4	5·3	2·2	17·9	1,862	1,757	146	133	91·1	9·1	2·2	4·9	2·0	15·5	2,062	1,412
Free flowering . . .	124	120	96·8	8·1	2·2	4·3	1·6	16·1	1,932	1,558	103	99	96·1	8·5	2·1	4·7	1·7	19·7	1,950	1,893

TABLE VII—*contd.*

Showing the result of pollination experiments in toria *and* sarson *with stigmas and pollen grains of different ages during the years 1930-31 and 1931-32*

Treatment	TORIA									SARSON (Brown-seeded)									
	Total number of flowers treated	Total number of pods treated	Percentage of pod-setting	Average length in cm. per pod				Average number of normal seeds per pod	Total No. of normal seeds	Average No. of normal seeds calculated per 100 flowers	Total number of flowers treated	Total No. of pods formed	Percentage of pod-setting	Average length in cm. per pod				Average No. of normal seeds per pod	Total No. of normal seeds	Average No. of normal seeds calculated per 100 flowers
				Entire pod	Pedicel	Seed-bearing portion	Beak							Entire pod	Pedicel	Seed-bearing portion	Beak			
One day old pollen and fresh stigma	112	111	99·1	8·4	2·1	4·9	1·4	12·6	1,399	1,249	109	109	100·0	9·7	1·9	5·9	1·9	19·5	2,126	1,950
Two days old pollen and fresh stigma.	125	121	96·8	8·6	2·0	5·0	1·6	13·8	1,670	1,336	117	116	99·1	9·6	1·9	5·7	2·0	17·5	2,030	1,735
Three days old pollen and fresh stigma	109	108	99·1	9·4	2·2	5·5	1·7	18·9	2,041	1,872	115	111	96·5	9·7	1·7	6·0	2·0	17·4	1,931	1,679
Four days old pollen and fresh stigma	120	116	96·7	8·9	2·2	4·8	1·9	19·8	2,297	1,906	97	94	96·8	8·5	1·8	4·7	2·0	15·1	1,419	1,463
Five days old pollen and fresh stigma	108	99	91·7	8·7	2·1	4·6	2·0	16·6	1,643	1,521	108	101	93·5	8·7	1·8	4·9	2·0	14·0	1,414	1,309
Six days old pollen and fresh stigma	82	82	100·0	8·2	1·7	4·7	1·8	10·0	820	1,000	47	44	93·6	9·6	1·7	5·0	1·9	12·7	559	1,189
Seven days old pollen and fresh stigma	86	82	95·4	8·3	1·6	4·8	1·9	12·2	1,000	1,163	37	29	78·4	7·0	2·0	3·8	1·2	10·0	290	784
Eight days old pollen and fresh stigma	72	58	80·6	5·6	1·3	3·3	1·0	7·4	429	596	41	40	97·6	8·9	2·5	4·5	1·9	9·8	392	956
Nine days old pollen and fresh stigma	65	50	76·9	4·6	1·3	2·4	0·9	2·2	110	169	47	45	95·7	6·6	1·6	3·6	1·4	9·4	423	900
Fresh stigma and fresh pollen .	147	143	97·3	8·8	1·9	5·3	1·6	16·2	2,317	1,576	102	99	97·1	9·2	2·0	4·9	2·3	16·5	1,634	1,602
Free flowering . . .	178	174	97·8	7·3	1·8	4·1	1·4	16·1	2,801	1,574	141	141	100·0	8·0	2·1	4·7	1·2	17·3	2,439	1,730

One day old stigma and one day old pollen	50	49	98·0	8·6	2·2	4·6	1·8	13·0	637	1,274	32	32	100·0	7·9	2·3	3·9	1·7	12·0	384	1,200
One day old stigma and two days old pollen	44	42	95·4	9·2	2·3	5·5	1·4	14·1	592	1,345	26	26	100·0	8·4	2·2	4·2	2·0	16·0	416	1,600
One day old stigma and three days old pollen	37	34	91·9	9·3	2·1	5·4	1·8	16·1	547	1,479	26	25	96·1	8·1	2·2	4·1	1·8	14·0	350	1,346
Two days old stigma and one day old pollen	47	47	100·0	8·0	2·0	4·9	1·1	16·9	794	1,689	32	31	96·9	7·7	2·0	3·8	1·9	17·2	533	1,666
Two days old stigma and two days old pollen	46	46	100·0	8·5	2·1	5·0	1·4	14·4	662	1,439	35	35	100·0	7·1	1·9	3·4	1·8	14·0	490	1,400
Two days old stigma and three days old pollen	46	46	100·0	8·3	1·9	5·0	1·4	16·9	777	1,689	32	32	100·0	7·2	1·9	3·8	1·5	18·2	582	1,819
Three days old stigma and one day old pollen	24	24	100·0	9·9	2·3	5·6	2·0	15·	70	1,541	35	35	100·0	6·6	1·8	3·4	1·4	14·3	501	1,431
Three days old st ,d two days old pollen	25	25	100·0	9·3	2·1	5·4	1·8	16·7	418	1,672	35	35	100·0	7·3	1·9	3·6	1·8	17·0	595	1,700
Three days old stigma and three days old pollen	24	22	91·7	9·7	2·4	5·3	2·0	15·3	337	1,404	30	30	100·0	6·0	1·7	2·9	1·4	10·5	315	1,050
Fresh stigma and fresh pollen .	111	109	98·2	9·2	2·1	5·2	1·9	18·0	1,962	1,768	32	31	98·8	7·4	1·9	3·6	1·9	13·6	1,102	1,344
Free flowering . . .	85	85	100·0	8·1	2·5	4·4	1·2	17·4	1,479	1,740	60	60	100·0	7·4	2·6	3·6	1·2	17·4	1,044	1,740

V. Breeding of improved varieties of *toria* and *sarson*.

(*A*) *Evolution of unit species*

From the results of self and cross fertilization experiments mentioned above it is natural to expect that inbreeding (self-fertilization) in *toria* and brown-seeded *sarson* would result in loss of vigour. This has actually been observed to be the case. The plants raised from selfed seed were usually found to be short statured and much less vigorous than those raised from seeds obtained by crossing. Actual determinations were made in 1931-32 with regard to the height of plants, number of branches, and percentage of flowers that set into pods in the plants raised from seeds obtained from selfing and crossing. The average results are given below :—

Name of crop	Seed obtained by	No. of plants studied	Average height of plants in cm.	Average number of primary branches per plant	Average percentage of pod-setting
Toria . .	Selfing . . .	50	93·4	9·4	41·4
	Crossing . .	100	109·4	11·3	46·7
Brown-seeded *sarson*	Selfing . . .	50	90·5	7·4	39·8
	Crossing . .	100	112·5	10·5	50·2

Thus it is evident that the plants raised from seeds obtained by crossing attained greater height, produced more branches, and formed greater number of pods than those raised from selfed seed. No determinations were made as regards the size of pods, weight of seeds and number of seeds per pod. But from a general examination of the material it was quite apparent that in these respects too the plants raised from seeds obtained by crossing scored over those raised from selfed seed. Many of the seeds obtained by natural selfing did not germinate at all.

In light of the above it will be seen that, in *toria* and brown-seeded *sarson*, pure lines, though they can be produced by continued (enforced) inbreeding, have, as such no utility for the farmer. In the case of yellow-seeded *sarson*, however, which is self-fertile, a large number of unit species or types have been isolated from the mixtures commonly grown in some parts of the province, some of which are very promising.

(*B*) *Mass selection and the factors concerned*

From the facts mentioned above it is clear that crossing in *toria* and brown-seeded *sarson* is a sure means of obtaining high yields, which in the breeding of improved varieties should not be excluded. Crossing should, however, be limited

to certain desirable forms, so that some sort of group breeding or mass selection should be resorted to in order to effect improvement in these crops. Mass selection consists of selecting such plants as possess the desirable attributes. By following this method considerable improvement of these two crops has already been effected in the Punjab. This is borne out by the following facts regarding the work done on *toria* crop. To start with, it may be noted that in the case of *toria* crop, the desirable or choice plants should for various reasons possess the following attributes.

1. They should be fairly early so that growth of plants and development of fruit may be completed before the season gets too cold and frosty. Uniformity in maturing is essential to avoid difficulties in harvesting.

2. Habit should be profusely branching, with branches ascending so that the form of the plant should be more or less like that of an inverted cone. Plants having wide, open tops or those having few branches should be discarded. The former occupy too much space for the yield they give.

3. The branches should possess the capacity of profuse podding.

4. Pods should be long, containing bold seeds, as bolder seeds contain a higher percentage of oil. Relative weight of seeds would be an indication of their boldness and it is interesting to note that the colour of freshly harvested seeds in *toria* and brown-seeded *sarson* has been found to be correlated with their weight. This correlation in addition to having a commercial utility can serve a very useful purpose in selection work. A sample of seeds of *toria* and brown-seeded *sarson* obtained from one and the same plant usually contains two differently coloured types of seeds, *viz.*, light brown or reddish and deep brown or blackish. A comparison between the weight of these blackish and reddish seeds (Table VIII) shows that red seeds weigh less than the black seeds. On the average one hundred blackish seeds weigh about 21 per cent and 35 per cent higher in *toria* and *sarson* respectively than the reddish seeds. This difference is probably due to the difference in nutrition as it has been found that both in *toria* and *sarson* black or blackish seeds predominate in the basal portions of the branches. This predominance is in case of *toria* reversed towards the middle of a branch and further on, the predominance of red or reddish seeds becomes greater and greater until at the top there are about 3 of them to 1 of black or blackish seeds. But, in *sarson* it goes a long way up and it is only at the tip that it suffers a diminution. During the year 1932-33 there was frost during the seed development period of *toria* and development of seeds was consequently adversely affected. It was found that the produce contained an exceptionally large number of reddish seeds This fact also lends support to the assumption that development of colouration is associated with nutrition. A point of still greater interest from the breeder's point of view is the fact that the *toria* plants raised from blackish seeds have been found to grow taller, bear more pods, yield comparatively heavier seeds and give a higher yield per plant than those raised from the reddish seeds.

TABLE VIII

Comparing the weight of black and red seeds in toria *and* sarson, *1927-28*

Plant No.	*Toria*			*Sarson*		
	Weight in grams per 100 seeds			Weight in grams per 100 seeds		
	Black	Red	Difference in favour of black seeds	Black	Red	Difference in favour of black seeds
1 . . .	0·340	0·262	0·078	0·567	0·378	0.180
2 . . .	0·347	0·330	0·017	0·471	0·352	0.119
3 . . .	0·445	0·365	0·080	0·366	0·295	0.071
4 . . .	0·331	0·215	0·116	0·357	0·295	0·062
5 . . .	0·355	0·310	0·045	0·416	0·301	0·115
6 . . .	0·370	0·265	0·105	0·427	0·332	0·095
7 . . .	0·351	0·317	0·034	0·397	0·269	0·128
8 . . .	0·330	0·295	0·035	0·414	0·295	0·119
9 . . .	0·367	0·315	0·052	0·383	0·299	0·084
Average. .	0·359	0·297	0·062	0·422	0·313	0·109
In terms of 100 . .	121	100	21	135	100	35

Determinations made in 1931-32 with regard to these characteristics are as follows :—

Progeny of	No. of plants studied	Average height per plant in cm.	Average No. of primary branches	Average No. of pods per plant	Average weight of 100 seeds in grms.	Average yield per plant in grms.
Blackish seeds	100	168	11	846	0·235	33·56
Reddish seeds	100	163	11	735	0·220	26·44

Thus it is apparent that the plants raised from the blackish seeds give about 27 per cent more yield per plant than those raised from reddish seeds. Selection of plants on the basis of high percentage of blackish seeds would therefore be a decided advantage.

It may also be mentioned that the yield per plant in *toria* has been found to be more or less positively correlated with the number of primary branches, length of pods, and weight of 1000 seeds. The correlation worked out from the data obtained in the year 1932-33 are as follows :—

Characters related	No. of pairs of values used	$R \pm$P. E.	Correlations
No. of primary branches and yield per plant	190	$+0{\cdot}63+0{\cdot}029$	Very high, positive, significant
Length of pods and yield per plant	181	$0{\cdot}39\pm0{\cdot}042$	High, positive, significant
Weight of 1000 seeds and yield per plant	147	$\pm0{\cdot}18\pm0{\cdot}054$	Low, positive, not significant

Considering the various results quoted above, it is evident that in order to obtain high yielding selections profusely branched plants with longer pods and comparatively bold seeds, majority of the seeds being blackish in colour, should be selected. On the basis of these considerations the selection work on *toria* and *sarson* crops has been carried out in the Punjab during the past several years and the selected strains have invariably been giving one to two maunds more yield per acre than the unselected strain.

(*C*) *Hybridization*

A number of intercrosses were attempted between *toria* and *sarson* and between them and some other Brassicae. The results of some of the earlier studies [Ali Mohammad, Singh and Alam, 1931] showed that the different species of the genus Brassicae referred to above crossed readily with one another and the pods and seeds formed as a result of these crosses were quite normal as compared with pods and seeds formed on free flowering branches of the seed parent in each case. The investigations on these crosses have to be continued further for a number of years before any definite conclusions can be drawn. Reference is, however, made to a few important points which have come to light as a result of some of these studies and have added greatly to the results of the pollination studies referred to above, in so far as the improvement of the two crops under

consideration, *viz.*, *toria* and *sarson*, is concerned. From the results of the various pollination experiments it has already been pointed out that the common brown-seeded forms of *toria* and *sarson* are high self-sterile giving very poor setting under bag and are extensively cross pollinated in nature, depending almost entirely on insects for their pollination. Yellow-seeded form of *sarson*, on the other hand, is self-fertile, giving very good setting under bag. The yellow-seeded form of *sarson* has been intercrossed reciprocally with both *toria* and brown-seeded form of *sarson* in order to evolve strains which in addition to possessing all the desirable characteristics of *toria* and brown-seeded *sarson* should have the invaluable merit of being self-fertile like the yellow-seeded *sarson*. These studies are being pursued further but from the results so far achieved it is evident that self-fertility behaves as an inherited character independent of colour of seed and it has been found possible to obtain self-compatible plants having brown seeds like the common forms of *toria* and *sarson*. Therefore, the former idea that there is a close association between self-fertility and yellowness of seeds should be abandoned. The investigations into the various aspects of the problem connected with the inheritance of characters will form the subject for discussion in a separate paper but a special point of practical interest which has come to light as a result of these hybridization studies is the possibility of introducing self-fertility into the brown-seeded forms of *toria* and *sarson* referred to above. This would give a decided advantage as the self-fertile strains would not depend on insects for their pollination and therefore, the periodic failure of these crops due to insufficiency of insect visitors would entirely vanish. The evolution of highly improved types of plants involves the study of several other plant characteristics about the inheritance of which nothing definite can yet be stated. The line of work being followed is very hopeful, but the success will depend on how far the plants raised in the future generations show combinations of various valuable and economic characteristics. There is no reason to doubt that as a result of these studies, there is a great possibility of the production of entirely new forms of *toria* and *sarson* superior to those now in existence, but the evolution of such highly improved strains is a matter of gradual selection by a series of steps. It is particularly note-worthy that as a result of the studies referred to above in this paper, abundant material has now become available which is sure to yield results of far greater value than those already obtained.

VI. Summary

1. *Toria* and *sarson*, for which the botanical names *Brassica napus L.* var. *dichotoma* Prain, and *Brassica campestris* L. var. *sarson* Prain, have been used in this paper on the authority of Prain, are by far the most important oilseed plants of the Punjab.

2. In both of them, the floral mechanism is such that it provides few chances for natural selfing. Under bags they are shy seed-setters. On an average of six years, only 12·3 and 20·3 per cent, respectively, of *toria* and *sarson* flowers formed

pods under such conditions and many of these pods were pods in name only with their seed bearing portions greatly reduced.

3. Yields of *toria* are adversely affected if there occur in November long spells of cloudy, humid, rainy weather. This is due to the fact that insects do not come out on such days to pollinate the flowers. *Andrena ilerda*, *Apis florea*, and *Halictus sp.* are the chief insect pollinators of *toria* and *sarson*.

4. Artificial selfing in *toria* and brown-seeded *sarson* by hand-pollination, at the time when flowers open, though it hardly increased the number of normal seeds per pod, makes some increase in the number of pods set. This increased production remains far short, however, of that on the free-flowering branches open to the visits of insects.

5. From crossing under bag and getting even a better setting than on free flowering branches, combined with the fact that artificial selfing does not improve matters much, it is concluded that the high amount of self-sterility prevailing in these two plants is not due to external causes alone, but due to internal ones (self incompatibility) also.

6. By self-pollinating a flower-bud two and three days, in the case of *toria* and three days in the case of brown-seeded *sarson*, before it opens out into a flower, quite a good pod-setting and seed production is obtained which compares favourably with that on free flowering branches.

7. Flowers self-pollinated two or three days after opening gave much better pod-setting and seed production than those selfed on the same day when flowers opened or a day after their opening.

8. The probable cause of self-sterility, as so far known, is the slow growth of 'self' pollen than of foreign pollen in the stylar tissue which is accounted for by an inhibiting action of that tissue. From the observations made it is argued that in *toria* and *sarson* this kind of inhibiting action is not equally effective in flowers of different ages and it appears more likely that in these plants this inhibiting action may be due mainly to the effect of a certain secretion produced actively in the stylar tissue between one to two days before and after the opening of the flowers.

9. Pollen grains in *toria* and *sarson* remain viable for at least 7 days after collection from the freshly opened flowers and the stigmas remain receptive for 3 days after the opening of flowers.

10. In both *toria* and *sarson* reddish coloured seeds weigh less than blackish coloured seeds. In the case of *toria*, number of primary branches, length of pods and weight of seeds have been found to be more or less positively correlated with yield per plant.

11. Owing to loss of vigour in *toria* and brown-seeded *sarson* by inbreeding, pure lines have no direct economic utility. To obtain improved varieties some sort of group breeding must be resorted to and considerable improvement has already been achieved by mass selection.

12. As a result of the hybridization studies in *toria* and *sarson* self-fertility is found to behave as an inherited character independent of colour of seed and self-compatible brown-seeded plants have been evolved. Abundant material has become available which shows the possibility of evolving entirely new self-compatible forms of *toria* and *sarson* superior to those now in existence.

REFERENCES

Akhtar, A. R. (1932). *Ind. J. Agric. Sci.* **2**, 280.

Ali Mohammad; Singh, R. D.; and Alam, Z. (1931). *Ind. J. Agric. Sci.* **1**, 109-136.

Balls, W. L., Templeton, J., Brown, C. H. and Kilani, M. (1929). *Bull. Ministry Agric. Egypt* No. **89**, 1-27.

Burkill, I. H. (1906, 1). *J. and Proc. Asiatic Soc. Bengal* **2**, 511-514.

——————— (1906, 2). *J. and Proc. Asiatic Soc. Bengal* **2**, 515-520.

——————— (1906, 3). *J. and Proc. Asiatic Soc. Bengal* **2**, 521-525.

——————— (1907). *J. and Proc. Asiatic Soc. Bengal* **3**, 517-526,

——————— (1908). *J. and Proc. Asiatic Soc. Bengal* **4**, 179-231.

——————— (1911). *J. and Proc. Asiatic Soc. Bengal* **6**, 101-107.

Chittenden, F. J. (1914). *Ann. App. Biol.* **1**,

Farrar, C. L. (1931). *J. Econ. Ent.* **14**,

Fox Wilson, G. (1929). *Ann. App. Biol.* **16**.

Hendrickson, A. H. (1916). *Calif. Agric. Expt. Stn. Bull. No.* **274**, 127-132.

Hutson, R. (1926). *New Jersey Agric. Expt. Stn. Bull.* **434**.

Hooper, C. H. (1923). ' The value of hive and wild bees in the production of fruit '.

Howard, A. Howard, G. L. C. and Abdur Rahman Khan (1910). *Mem. Dept. Agric. Ind., Bot. Ser.* **3**, No. 6.

Jost, L. (1907). *Uber die Selbst-sterilitat einiger Blutn. Bot. Ztg.* **65**.

Kakizaki, Y. and Kasai, T. (1933). *J. Hered.* **24**, 359-360.

Lewis, C. I. and Vincent, C. C. (1909). *Ore. Agric. Exp. Stn. Bull.* **104**.

Martin, J. N. (1913). *Bot. Gaz.* **56**.

Morris, O. M. (1921). *Washington Agric. Exp. Stn. Bull.* **163**, 1-32.

Nelson, (1927). *J. Gen.* **19**.

Overholser, E. L. (1927). *Calif. Agric. Exp. Stn. Bull.* **426**, 1-17.

Pammel, L. H. (1923). *Iowa Rept. State Apiarist* 1922, pp. 51-60, Iowa, 1923 (R. A. E. A. 1924 p. 18).

Phillipe, E. F. (1930). *J. Econ. Ent.* **23**, 218-223.

Prain (1898). *Agric. Ledger* **1**.

Silow (1931). *Welsh P. B. Station, Series H, No.* **12**, 1921-1930.

Treherne, R. C. (1923). *15th Ann. Rept. Quebec. Soc. Pro. Plants*, 1922-23, pp. 47-59, Quebec 1923 (R. A. E. A. XI, p. 477).

Walton, C. L. (1927). *Ann. App. Biol.* **14**, 465-469.

Williams, R. D. (1925). ' Studies concerning the pollination, fertilization and breeding of red clover. '

Branched and normal ear-heads from one F_2 plant in a cross between Chevalier and Pusa Type 21 barleys.

STUDIES IN INDIAN BARLEYS'

3. BRANCHED EARS IN BARLEY AND THEIR MODE OF INHERITANCE

BY

R. D. BOSE, B.Sc.,

Officiating Assistant Economic Botanist, Pusa

(Received for publication on 4th December 1934)

(With Plate X)

A small number of plants with branched earheads were observed in 1931-32 in the F_2 population of a cross between Chevalier (2-rowed) and Pusa Type 21 (6-rowed) barleys. The branching was invariably restricted to the lower half of the ear and only a few ears and not all exhibited this peculiarity in each plant. Plate X depicts a branched earhead as well as a normal unbranched ear from one and the same plant.

Branched ears of barley occur but very rarely in nature and indeed only a very few references exist about this phonomenon. In 1910 Tschermak recorded a case of inheritance of branched ears in barley and found that segregation for this character was on a monohybrid basis, the branched ear type being the recessive phenotype. Schneider [1913] described a new form of barley, which appeared in Posen in 1902, the chief feature of which was branching of the spikes—a characteristic which proved to be transmitted with constancy. Although this type of barley had a somewhat higher yielding capacity than ordinary varieties, the quality was said to be decidedly inferior. Schneider has also given a few interesting references some of which are quoted below.

"In 1671 a note was published mentioning the fact that a branched ear of barley which consisted of 15 larger and 9 smaller sized ears was found in 1637 in the country of Glatz in Silesia on a field which for a long time had been uncultivated, it was brought to the Emperor and engraved in stone". Koernicke [1882] and Wittmack [1871] have also studied the peculiar appearance of branched ears. The latter holds that branching is not only caused by specially rich conditions of soil but partly also by abnormal weather conditions—great moisture in the spring or cold in the preceding winter. Koernicke, on the other hand, thinks that the influence of moisture and cold is not great, otherwise rye in the north would shew a more frequent occurrence of branched ears. He believes it possible however that an unfavourable winter, in which the plants are partly frozen, may cause a branching of the ears.

Rimpau [1899] found an individual plant with branched ears among 2-rowed barleys and made the type constant by selection (*Lander. Jahrb.* 1891, D. L. Pr. 1899. Nr. 79). A breeding experiment with branched barley with the intention of creating a marketable product was undertaken in 1900 at the Seed Breeding Institute, Aderstedt and is mentioned in an article by Hoffmann of Lichinia (in D L. Pr. 1903, Nr. 3, page 17). The original material was a branched individual plant belonging to a two-rowed drooping barley. It was assumed that it arose as a spontaneous variation. Herr Antsrat Wessling in Westeregeln bred a Chevalier barley which had a branched form (Fig. in Schonfeld, "Brangersten in Bilde" Verlag Paul Parey, Berlin). Schneider also reported the appearance of branched ears frequently in Goldthorpe barley and once in Groninger winter barley but believed that the character was not constant.

On the cause of the occurrence of branched ears the views of different people vary considerably but the phenomenon is mostly attributed to external influences. As mentioned above Koernicke and Wittmack assigned the change to soil and weather conditions, Kuhn of Halle (D. L. Pr. 1899, Nr. 79) believed in the influence of bacteria, while Rimpau assumed spontaneous variations. Hoffmann of Lichinia (D. L. Pr. 1903, Nr. 3) observed the branching during transformation of summer into winter forms. Tschermak [1910] holds that the branching of the ears occurs after previous hybridization and that wheat-rye hybrids show occasional luxuriant forms. Blaringhem [Siehe Fruwirth Zucht. Lander. Kultur pfl ; 4, 293] has succeeded in inducing branching of the axis by ' wounding '.

In wheat, branched ears have been recorded by many workers. Meunissier [1918] and Tschermak [1923] believed that the normal condition was dominant to the branched ear of *turgidum*, and that in F_2 the monohybrid type of segregation occurred. Nilsson-Leissner [1925] found some plants with branched ears among *spelta* individuals from the cross *spelta* × *vulgare*, the branched condition being recessive but dependent on several multiple genes. Biffen [1916] found branched ears among the offspring of the cross with normal types in *polonicum* × *vulgare*. Similarly Kezer and Boyack [1918] recorded branched ears in a cross between *vulgare* × *dicoccum* wheats.

Branched ears have also been observed in maize by Collins [1917] and by Kempton [1923] who reported that the character seemed to be recessive to the normal type of ear.

It is the purpose of this paper to describe the mode of inheritance of branched ears that has been observed at Pusa in a cross between Chevalier and Pusa Type 21 barleys.

Material and method

Chevalier barley was imported from England in 1927 and Type 21 was evolved by selection at Pusa in 1925 [Bose, 1931]. Since then both these types have been grown annually during the *Rabi* seasons, *viz.*, during the period October to March,

but not a single earhead with branched ears has upto now been observed in them. The cross between these two types was made in 1929-30 in order to study the inheritance of some characters in an interspecific cross between a 2-rowed (Chevalier) and a 6-rowed (Pusa Type 21) barley. Only two F_1 plants could be raised in the following year but neither of them showed any trace of this abnormality. When the F_2 generation of this cross was grown in 1931-32 it was observed for the first time that 21 plants in a total population of 374 showed some earheads with a branched nature. The extent of branching in individual ears varied considerably, three to five branches per ear being observed very frequently

All the plants with branched ears invariably had narrow and lax earheads with very poorly developed grains. A good deal of sterility also occurred in most cases and the hybrids exhibiting this phenomenon appeared to be neither good for quality nor for producing good yields. The only importance of these branched ear types, therefore, lies in their academic value, and they are not expected to yield results of any economic importance.

F_2 Generation

The F_2 progeny, along with the parental types, was grown in a field of average fertility. The distance between individual plants was one foot within and about three feet between the rows. The genetic study of the inheritance of characters such as fertility of the lateral spikelet, colour of the leaf-sheath and in the auricle, maturity, etc., will form the subject of another communication. The present paper is limited simply to the inheritance of branched ears. Segregation in F_2 was as follows :—

	Cross	No. of plants with		Total
		Normal ears	Branched ears	
	G-1	185	12	197
	G-2	175	9	184
Observed	..	360	21	381
Calculated on 15 : 1 . .	..	357·2	23·8	..
Deviation = 2·8 Dev. = 2·8 S. E. = 4·7 = 0·6.				

Therefore the fit between observed and expected frequencies was very close and indicated that duplicate factors were responsible for the inheritance of branched ears in this barley cross.

F_3 Generation

Thirty-four families of this cross were studied in the F_3 generation for this character. Three F_2 plants with branched earheads and thirty-one with normal ears were selected for this purpose. The progeny of the plants with branched ears bred true for this character and thus proved the recessive nature of this phenotype. Of the remaining 31 families, 17 gave families of from 61 to 84 plants all with normal unbranched ears, while 7 families gave approximately 15 normal : 1 branched and 7 gave approximately 3 normal : 1 branched. On a duplicate factor hypothesis the three types should have occurred in the proportions 14·46 : 8·27 : 8·27. Expectation was therefore closely realised. The behaviour of the F_3 families is shown below :—

Table I

F_3 breeding behaviour for the inheritance of branched earheads

No. of families	Nature of F_2 parent	F_3 behaviour	No. of families	
			Observed	Expected
31	Normal unbranched ears	Pure . . .	17	14·46
		Segregating—		
		3 : 1 . . .	7	8·27
		15 : 1 . . .	7	8·27
3	Branched ears .	Pure . . .	3	3

It will be noted that the discrepancy between the observed and expected numbers of families is not great. Table II shows the frequencies of normal and branched earheads in segregating F_2 families.

TABLE II

F_3 frequencies for normal and branched earheads

Family	No. of plants with		Total number of plants	χ^2	P
	Normal ears	Branched ears			
7 families segregating on 3 :1—					
G. 1-58	64	18	82	0·41	0·5
G. 1-129	38	15	53	0·31	0·6
G. 2-19	48	13	61	0·44	0·5
G. 2-51	57	17	74	0·16	0·7
G. 2-53	53	17	70	0·02	0·9
G. 2-61	51	14	65	0·42	0·5
G. 2-160	54	20	74	0·16	0·7
Total	365	114	479	0·37	0·5
7 families segregating on 15 : 1—					
G. 1-110	51	2	53	0·55	0·5
G. 1-120	73	4	77	0·15	0·9
G. 1-165	56	3	59	0·14	0·9
G. 2-30	58	5	63	0·31	0·6
G. 2-75	56	4	60	0·02	0·9
G. 2-146	63	4	67	0·01	0·9
G. 2-165	63	3	66	0·33	0·6
Total	420	25	445	0·30	0·6

From the foregoing it may be concluded that the F_3 studies confirm the F_2 hypothesis that duplicate factors are responsible for the inheritance of branched earheads in this cross of Chevalier × Pusa Type 21 barley.

F_4 Generation

In 1933-34 thirteen families of this cross were grown for the study of the F_4 generation. Six plants with normal ears from F_2 families breeding true for normal ears gave families of from 83 to 147 plants, all with normal ears. Two plants with branched ears from F_2 families breeding true for branched ears gave families of 146 and 150 plants, all with branched ears. Five plants with normal ears from segregating F_2 families, segregated in F_3, and also in F_4. Details are given in Table III.

Table III

F_4 frequencies for normal and branched earheads

Family	F_3 segregation	No. of plants with		Total number of plants	χ^2	P
		Normal ears	Branched ears			
G. 1-58-33 . . .	3 : 1	140	40	180	0·74	0·4
G. 1-58-91 . . .	3 : 1	104	37	141	0·12	0·7
G. 2-165-60 . . .	15 : 1	102	5	107	0·45	0·5
G. 2-165-49 . . .	15 : 1	91	8	99	0·57	0·5
G. 2-146-86 . . .	15 : 1	125	5	130	1·28	0·3

This study proves once more that duplicate factors only were responsible for the inheritance of branched earheads in the cross under consideration.

Relation of fertility with the branched type of ear

Chevalier barley belongs to the two-rowed species *Hordeum distichon* L. while Pusa Type 21 is a six-rowed type belonging to the species *H. vulgare* L. The two

forms therefore differ in the nature of the fertility of the lateral spikelets. The lateral spikelets in the two-rowed barleys remain abortive and thus there are only two rows of grains developing from the central spikelets. The lateral spikelets in the 6-rowed barleys, on the other hand, are fully fertile giving rise therefore to six rows of grains in the ear. When these two barleys are crossed, the F_1 plants show an intermediate type of fertility and the F_2 segregation is on a monohybrid basis. That the character of branched earheads in barley in this cross segregated independently of the factors responsible for the nature of fertility of the lateral spikelets was brought out by the occurrence of this phenomenon in all phenotypes for fertility in the F_2 generation. Thus of the 21 plants which exhibited this character of branched ears in F_2, six plants had ears of the 2-rowed type, four plants had ears of the 6-rowed type and the remaining eleven plants had ears of the intermediate type of fertility. This relation is shown in Table IV.

TABLE IV

Relation of fertility with the branched type of earhead in F_2

Cross	2-ROWED		INTERMEDIATE		6-ROWED		Total.
	Normal	Branched	Normal	Branched	Normal	Branched	
G. 1 . . .	51	4	88	6	46	2	197
G. 2 . . .	39	2	91	5	45	2	184
Total observed	90	6	179	11	91	4	381
Expected	89.30	6.95	178.60	11.90	89.30	5.95	..

$\chi^2 = 0.8306$ *P*—Between 0.95 and 0.98

The fit between observed and expected ratios on the basis of independent segregation is quite close. The fertility character segregates on a 1 : 2 :1 ratio while the earhead character shows a segregation on a 15 : 1 basis. This is further confirmed by the behaviour of the segregating families in F_3 as shown in Table V.

TABLE V

Relation of fertility with the branched type of earhead in F_3

Family	F_3 segregation for earhead character	2-ROWED		INTERMEDIATE		6-ROWED		Total
		Normal	Branched	Normal	Branched	Normal	Branched	
G. 1-110	15:1	13	0	26	2	12	0	53
G. 1-120	15:1	19	1	38	3	16	0	77
G. 1-165	15:1	12	1	31	1	13	1	59
G. 2-30	15:1	17	1	28	3	13	1	63
G. 2-75	15:1	16	0	28	2	12	2	60
G. 2-146	15:1	14	1	34	2	15	1	67
G. 2-165	15:1	16	0	29	3	18	0	66
Total observed		107	4	214	16	99	5	445
Expected		104.25	6.95	208.50	13.90	104.25	6.95	..
$\chi^2 = 2.3321$			$P = 0.80$	nearly				
G. 1-58	3:1	14	5	34	9	16	4	82
G. 1-129	3:1	11	4	17	7	10	4	53
G. 2-19	3:1	11	3	24	7	13	3	61
G. 2-51	3:1	13	4	30	10	14	3	74
G. 2-53	3:1	14	5	24	9	15	3	70
G. 2-61	3:1	15	2	24	8	12	4	65
G. 2-160	3:1	12	4	27	11	15	5	74
Total observed		90	27	180	61	95	26	479
Expected		89.82	29.94	179.64	59.88	89.82	29.94	..

$\chi^2 = 1 \cdot 1279$ P—between 0.95 and 0.98

Discussion of results

From the results of the foregoing studies it may be concluded that the production of branched earheads in this cross of barley depends on the interaction of duplicate factors and that the branched ear phenotype acts as the double recessive.

Type 21 barley has never been observed to throw out any branched earheads and neither has the strain of Chevalier barley grown at Pusa for a number of years, but Herr Antsrat Wessling (page 156) bred a Chevalier barley having a branched form.

In the present case let N_1N_1 be a factor which produces normal development of the barley ear and let N_2N_2 be another factor which operates in the same way as N_1N_1. The complete absence of both N_1 and N_2 will therefore produce branched earheads. The genetic interpretation of this cross may be shown in the Punnet-square given below :—

P_1 $N_1N_1n_2n_2$ (Chevalier) Normal × $n_1n_1N_2N_2$ (Pusa T. 21) rmal

F_1 $N_1n_1N_2n_2$ (F_1 hybrids) Normal

F_1 Gametes

F_2	N_1N_2	N_1n_2	n_1N_2	n_1n_2
N_1N_2	$N_1N_1N_2N_2$ 1 : 0	$N_1N_1N_2n_2$ 1 : 0	$N_1n_1N_2N_2$ 1 : 0	$N_1n_1N_2n_2$ 15 : 1
N_1n_2	$N_1N_1N_2n_2$ 1 : 0	$N_1N_1n_2n_2$ 1 : 0	$N_1n_1N_2n_2$ 15 : 1	$N_1n_1n_2n_2$ 3 : 1
n_1N_2	$N_2n_1N_2n_2$ 1 : 0	$N_1n_1N_2n_2$ 15 : 1	$n_1n_1N_2N_2$ 1 : 0	$n_1n_1N_2n_2$ 3 : 1
n_1n_2	$N_1n_1N_2n_2$ 15 : 1	$N_1n_1n_2n_2$ 3 : 1	$n_1n_1N_2n_2$ 3 : 1	$n_1n_1n_2n_2$ 0 : 1

It will be apparent from the above that only one-sixteenth of the population with the genotypic constitution $n_1n_1n_2n_2$ will breed true for branched earheads;

four-sixteenths with the genotypic constitution of $N_1n_1N_2n_2$ will segregate on a 15 : 1 basis; four-sixteenth with either $N_1n_1n_2n_2$ or $n_1n_1N_2n_2$ will segregate on a 3 : 1 basis, while all the other genotypes will bear normal, unbranched ears. All the expected genotypes have been realized in the cross under consideration.

As has been mentioned in the introductory chapter of this paper, the branching character was invariably restricted to the lower half of the ear and only a few ears and not all exhibited this peculiarity in each plant. If the genetic constitution of the plants producing branched earheads was such as to favour the development of branched ears alone, it may reasonably be argued as to why some ears only and not all should develop this characteristic. The difference between normal and branched ears in the same plant may possibly be attributed to differences in metabolism or to cytological disturbance at the growing point. In this connection it may be pointed out that Kempton [1923] observed in maize that the variability in branching cannot be attributed to the influence of hereditary modifying factors and must be considered as a phenomenon of expression rather than of transmission. This author impresses the view that the mode of inheritance of branched ears is characteristic of a large class of characters which seem so delicately balanced as to require some special conditions in ontogeny for complete expression and that internal developmental factors, quite aside from external influences, play a large part in the expression of structural characters. It may be acknowledged, however, that the absolute reason why all ears in the same plant do not develop this character of branched ears is a matter worth further investigation, and it is proposed in the future to study the matter from a cytological standpoint.

Summary

The inheritance of branched ears in barley was observed to depend on duplicate factors in a cross between Chevalier (2-rowed) and Pusa Type 21 (6-rowed) barleys. Neither the parents nor the F_1 showed this characteristic which made its appearance only in F_2 and the succeeding generations. The factors for branched ears segregated independently of the factors responsible for the inheritance of fertility of the earhead.

REFERENCES

Biffen, R. H. (1916) . . . *J. Gen. 5*, 225-228.
Bose, R. D. (1931) . . . *Ind. J. Agric. Sci. 1*, 58-89.
Collins, G. N. (1917) . . . *J. Agric. Res. 9*, 383-395,
Kempton, J. H. (1923) . . . *J. Hered. 14*, 243-251.
Kezer, A., and Blyak, B. (1918) . *Colorado Agric. Exp. Sta. Bull. 249*, 1139.
Koernicke, F. (1882) . . . *Z its. f. d. gesamte Brauwesen, Leipzig.*
Meunissier, A. (1918) . . . *Bull. Soc. Nat. Acclimat*, 1-31.
Nelsson-Leissner, G. (1925) . . *Hereditas. 7*, 1-74.
Rimpau (1899) . . . . *Lander. Jahrb.* 1891. D. L. Pr. 1899 Nr. 79.
Schneider, E. (1913) . . . *Z. Pflanzenz.* 1, 301-322.
Tschermak, E. (1910) . . . *Fruwirth. Zucht. Landw. Kulturpfl.* 4, 293.
——————— 1923) . . . *Fruwirth's Handbuchder Landwirt. Pflanz. Zuch. 4.*
Wittmack, L. (1871) . . . *Verh. d. bot. ver. d. Prov. Brandenburg. Jahrg.* S, *142.*

OIL FORMATION IN GROUNDNUT WITH REFERENCE TO QUALITY

BY

J. S. PATEL, M. Sc. (CORNELL), PH.D. (EDIN.),

Oil Seeds Specialist, Madras,

AND

C. R. SESHADRI, B.A., B.Sc. Ag.,

Assistant to the Oil Seeds Specialist

(Received for publication on 9th October 1934)

(With two text-figures)

I. INTRODUCTION

The Ottawa Trade Agreement has secured for India a preference of ten per cent. on the imports of Indian groundnut into the United Kingdom. The area under groundnut in West Africa has been increasing for the past ten years as a result of which competition between the West African and the Indian groundnut is getting keener.

Both the Empire Marketing Board and the Indian Trade Commissioner have drawn attention to the necessity of improving the quality of the Indian groundnut, particularly that of Madras. In point of view of quality the Indian groundnut must contain more of oil and less of free fatty acid than it does at present. The Empire Marketing Board has investigated the free fatty acid content of the samples of Indian groundnuts collected from the shipments to Europe during the two months, December 1926 and January 1927. A summary of the results of the investigations of the Empire Marketing Board is given in Table I.

TABLE I

Free fatty acid content of Indian groundnut kernels on arrival at European ports

Ports of shipment	December 1926 shipments*		January 1927 shipments*	
	Average free fatty acid content per cent	Range of free fatty acid content per cent	Average free fatty acid content per cent	Range of free fatty acid content per cent
Madras (*a*)	4·21	1·09—9·06	4·13	1·0—10·2
Pondicherry (*a*)	2·52	1·83—3·82	4·10	1·4— 8·7
Vizagapatam (*a*)	2·55	0·96—2·86	3·00	1·8— 3·2
Bimlipatam (*a*)	1·70	0·90—3·16	..	..
Coconada (*a*)	0·98	0·93—1·08	..	..
Cuddalore (*a*)	..	..	9·32	7·9—11·1
Negapatam (*a*)	..	..	8·44	5·0—11·6
Bombay (*a*)	1·32	0·65—2·26	1·55	0·9— 2·2
Murmagoa (*a*)	1·87	0·99—2·22	1·42	0·7— 2·6
Calicut (*a*)	7·94	3·60—12·25	5·00	3·8— 6·5
Bombay (*b*)	0·83	0·48—1·77	1·33	0·7— 3·0
Murmagoa (*c*)	2·25	0·93—3·09	1·77	1·6— 1·9

(*a*) Groundnut from Madras
(*b*) Groundnut from Bombay
(*c*) Groundnut from Khandesh

* The shipments in December 1926 and January 1927 amounted to 33,750 tons and 49,700 tons, respectively.

The Indian Trade Commissioner, London, remarks "If you take the Madras kernels as containing on an average four per cent of free fatty acids, you have a charge of about £2-10 per ton of oil for extraction and a loss of £14 in the sale of free fatty acids, as compared with the price obtained from the refined oil. Taking Madras exports at 300,000 tons of kernels annually, the cost of extracting free fatty acid amounts to about £300,000 and the loss compared with refined oil adds another £84,000 making nearly £400,000 lost in all".

An investigation into the causes which affect the quality of groundnut was therefore, taken up. The present investigation deals with the rate of oil formation in groundnut and the effect of early harvest on the quality of the produce. The effect of storing damp or moist kernels is under investigation and will form the subject of a separate paper.

II. Materials

The groundnut is a self-fertilized crop. The flower buds which are in the calyx in the evening, emerge by degrees and in the early morning the anthers burst and pollinate the stigma. Fertilization is complete before midday is past; the flower droops in the evening and withers completely in three days. The stalk of the ovary elongates and the gynophore is visible in about five to seven days after fertilization. The geotrophic gynophore, with the ovary, at its tip pierces the ground to a depth of two to five centimetres, and the ovary which has remained dormant till then begins to develop. This process requires two or three weeks after the opening of the flower, depending upon the position of the flower in the axis. For complete development of pod, it takes about sixty days from the time of fertilization.

A bunch groundnut of the Spanish type, locally known as Gudiyattam bunch, was utilized in the present investigation. About 10,000 flowers were marked by wire wickets. To mark the flowers blossoming on different dates, differently coloured wickets were used. After the 'pegs' (gynophore) began to appear, the threads which were tied to the flower were transferred to the respective gynophores. As the number of pegs that could be marked in this way was small and as the maximum percentage of fertilization was only forty-two, pegs showing similar state of development were also tagged to provide requisite material of uniform development. It was soon discovered that the kernels obtained from the flowers which opened on the same day were not uniform in their development since the pegs which developed nearer the ground entered the earth earlier and therefore produced kernels of relatively greater maturity. It was, therefore, found necessary to select those kernels which exhibited the same degree of maturity from the material harvested at various intervals.

The pods were harvested for the first time on the twenty-fifth day after flowering and the harvests were continued at intervals of a week up to the sixtieth day after flowering. Corresponding to these six harvests, six distinct stages in the development of the pods were obtained. A brief description of each of these six stages is given below:

Stage 1.—Pods obtained on the twenty-fifth day after flowering have a size of 0·5 to 1·5 cm. Shell is very fleshy and contains plenty of moisture. Kernels 2 to 3 mm. long are embedded in the fleshy mass. "Beak-end" kernel has not yet been formed.

Stage 2.—Pods on the thirty-second day after flowering are 0·75 to 2 cm. long but the shell is still fleshy. The kernels of one-seeded pod measure about 0·75 cm. in length. The " beak-end " kernel has just developed.

Stage 3.—Pods on the thirty-ninth day after flowering measure 1 to 2 cm. in length. The " stalk-end " portion of the shell is no longer fleshy but the " beak-end " portion is still fleshly. Kernels show traces of pigmentation. The " stalk-end " kernel in the two-kernelled pods is 0·75 to 1 cm. in length. The " beak-end " kernels are 0·3 to 0·5 cm. in length.

Stage 4.—Pods harvested on the forty-sixth day are from 1 to 2·5 cm. in length. Air space is observed between the kernel and the shell. Shells show papery lining. " Beak-end " kernels show traces of pigmentation and they have developed up to a length of 0·75 cm.

Stage 5.—Pods on the fifty-third day after flowering have developed up to 1 to 3 cm. in length. The air space between the shell and the kernels is increased. The white papery lining gradually disappears. The pigment on the kernel is marked.

Stage 6.—The lining of the shells of pods on the sixtieth day after flowering is discoloured. Kernels have hardened and the pigmentation on the testa is intensified.

The pods were removed to the laboratory immediately after harvest and washed and were dried by gently rolling them in a blotting paper.

III. Shelling percentage

The pods were shelled and the shelling percentages were determined for fresh materials before drying. The shelling percentage is expressed as the weight of kernels when the weight of the whole pods is taken as hundred.

From Table II where the shelling percentages for various stages of development are given, it is clear that the kernel develpes most rapidly between the thirty-ninth and forty-sixth day after flowering. Before and after that period the rate of increase in the shelling percentage is comparatively slow (Fig. 1).

Table II

Shelling percentages at various stages

Stage	2	3	4	5	6
No. of days after flowering .	32	39	46	53	60
Shelling percentage . .	6·6	17·3	54·9	66·3	72·9

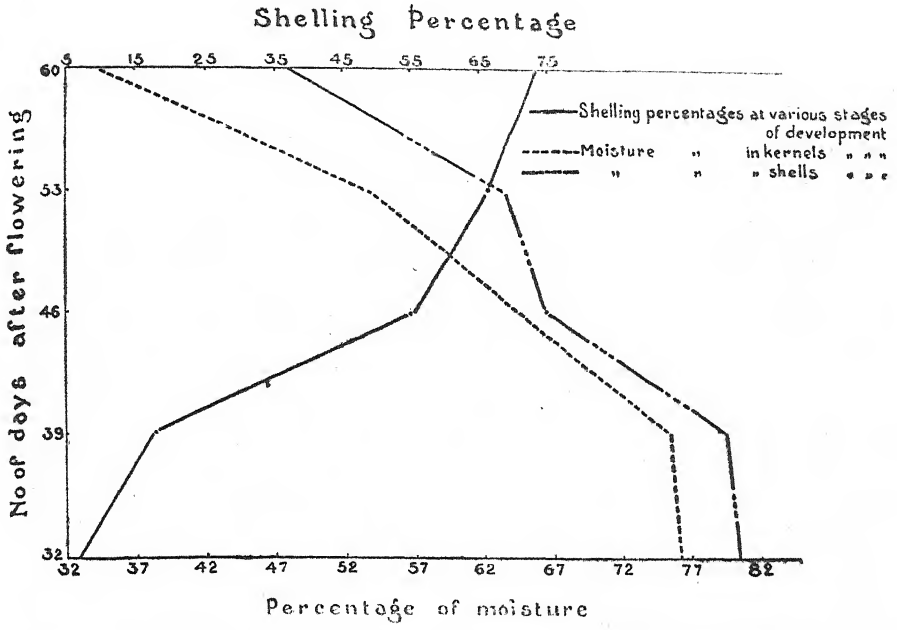


Fig. 1. Shelling percentage

IV. Moisture content

The moisture content of shells and kernels was determined by drying the samples in an oven at 98°C. in partial vacuum. This method does not give an indication of the absolute moisture content but it gives sufficient information to enable relative comparison.

In Table III the percentage of moisture in shells and kernels at various stages of the development are shown. It is observed that, in all the stages, the moisture content is higher in shells than in kernels. This indicates that a moisture gradient exists between the shells and the kernels, and that the transfer of moisture from the shells to kernels goes on throughout the development of the pod. The moisture content of both the shells and the kernels gets reduced as the pods mature (Fig. 1). That even mature kernels contain as high as thirty-three per cent. moisture is a fact which should not be lost sight of.

TABLE III

Percentage of moisture at various stages

Stage	2	3	4	5	6
No. of days after flowering	32	39	46	53	60
Moisture in shells	80·4	79·4	66·1	63·2	48·1
Moisture in kernels	76·2	75·5	65·0	53·9	33·9

V. OIL CONTENT

The oil content was determined for both the fresh and dried kernels. For the latter the material obtained after determining the moisture content was utilized. The oil was extracted with petroleum ether, using glass soxhlet apparatus and fat-free thimbles. The first extraction was taken to have been complete after the ether had been siphoned seven times. After completely drying the thimble containing the material, in an oven, the partially extracted material was re-ground and re-extracted until six siphonings were complete. The process was throughout controlled by duplicate samples.

It is evident from Table IV that the synthesis of oil goes on continuously and that both in the beginning and the end it is rather slow. The most rapid accumulation of the oil takes place during the fortnight commencing with the thirty-second day after flowering (Fig. 2).

TABLE IV

Percentage of oil in kernels at various stages

Stage	1	2	3	4	5	6
No. of days after flowering	25	32	39	46	53	60
Oil in fresh kernels	0·35	3·58	5·87	8·35	19·75	30·94
Oil in dried kernels	4·27	12·31	23·48	38·28	43·86	48·51
Rate of increase in oil per week	..	8·04	11·17	14·80	5·58	4·65

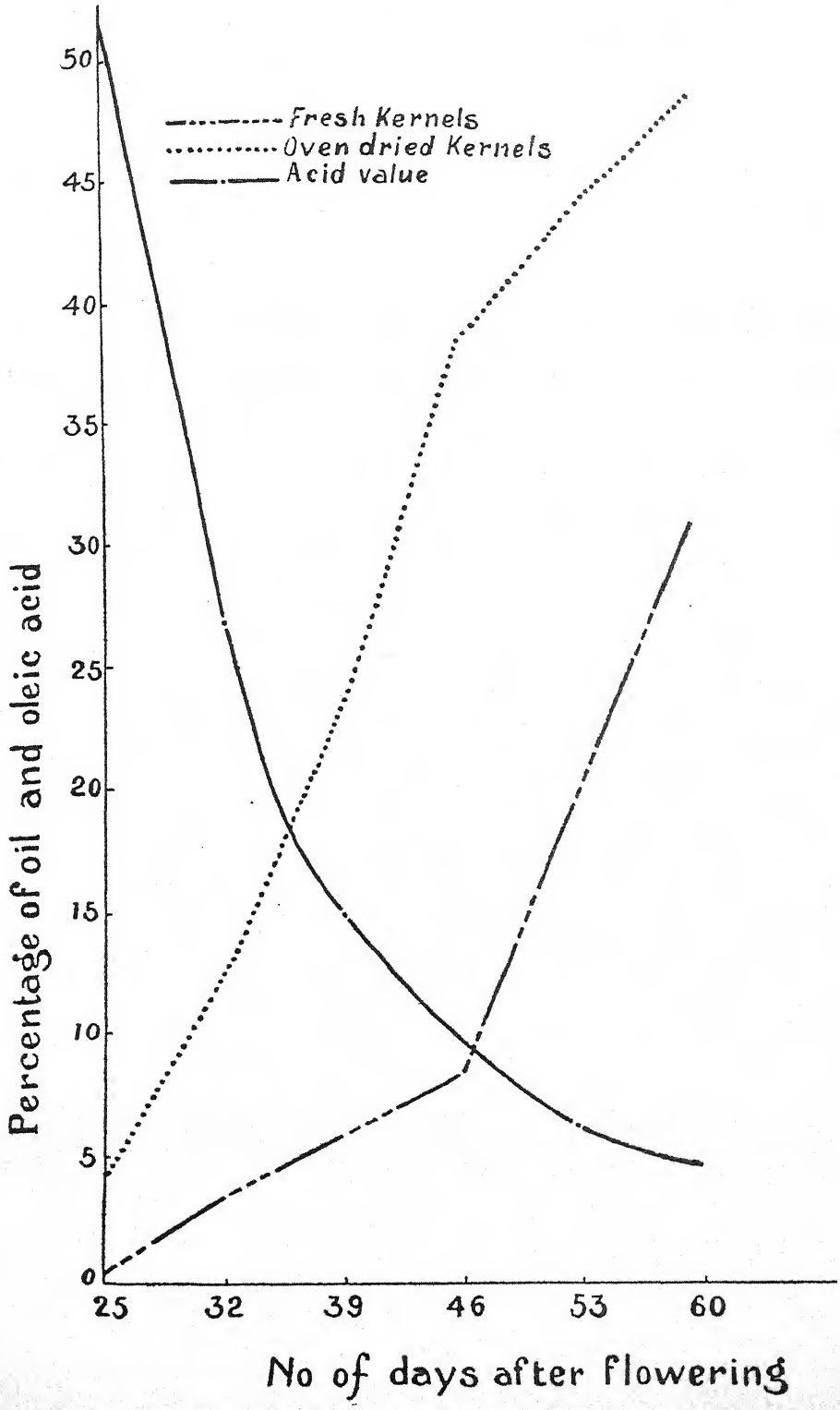


Fig. 2. Percentage of oil and acid value at different stages of groundnut kernels

VI. Acid value

To the flask containing oil extracted from the fresh sample, about 20 c.c. neutral 95 per cent. alcohol were added, and the flask immersed in a water-bath and agitated at intervals so as to dissolve the free fatty acids completely. The hot fluid was titrated with $N/10$ sodium hydroxide using phenolphthalein as indicator. The results have been expressed as percentage of oleic acid in 100 grms. of oil taking one c.c. of $N/10$ sodium hydroxide as equal to 0·028 grm. of oleic acid.

In Table V the acid value of oil at various stages of development of the seed is given. The figures show that the percentage of free fatty acid decreases as the seed matures. This reduction of the free fatty acid is more rapid during the twenty-fifth and thirty-ninth day after flowering (Fig. 2).

Table V

Free fatty acid content in kernels

Stage	1	2	3	4	5	6
No. of days after flowering .	25	32	39	46	53	60
Acid value expressed as oleic acid	51.45	26.62	14.72	9.87	6.28	5.07
Reduction in the acid value per week	..	24.83	11.90	4.85	3.59	1.21

VII. Discussion

Eyre [1931] has shown, in the case of linseed, that the oil accumulates rapidly during the early stages of the development of the seed after which further increase is comparatively slight. In the case of groundnut, however, the accumulation of the oil in the seed is rather slow both in the initial and the final stages of the development of the seed. Sahasrabuddhe and Kale's [1933] investigations on niger seed show : (*a*) that the formation of oil is most rapid in the early stages, (*b*) that the oil is formed from carbohydrates which are converted into free fatty acids by some enzymes, and (*c*) that the oil is transformed from the accumulated free fatty acids by enzymes.

The results of the present investigation are in agreement with the view that, in initial stages in the development of the seed, there is preponderance of the free fatty acid and that, in the final stage, the quantity of free fatty acid is considerably reduced and the quantity of oil is increased.

In Madras, there are two main cultivated varieties of groundnut, *viz.*, 'Local Mauritius' and 'Gudiyattam bunch'. The former ripens in about 130 days after

sowing and the latter in about ninety-five days. This period varies to the extent of fifteen to twenty-five days, depending on the soil and climatic conditions. Both the varieties flower in three to four weeks after sowing and the flowering continues till about three to four weeks before the harvest. It is, therefore, natural to find, at the time of harvest, a number of undeveloped pegs which have not entered the soil or entered the soil just prior to the harvest. At the time of the harvest, the pods are found to be in different stages of maturity, some entirely immature, while the others are partially mature. The produce therefore consists of a majority of mature kernels and the remaining partially mature or immature kernels which reduce the percentage of oil from the sample and increase the free fatty acid content. In Table VI the results of counts from 500 plants, of undeveloped pegs, tender nuts, immature nuts, and mature nuts of the two varieties are given. The figures are the averages of 500 plants pulled out at the time of harvest in different years, and these illustrate the proportion of immature kernels in the bulk produce.

TABLE VI

Results of '500 plant studies' in groundnut varieties 1930-33 rain-fed seasons

(Averages of 500 plants pulled out and counted at harvest time)

Name of variety	Year	Unde-veloped pegs	Tender nuts	Immature nuts	One-ker-nelled pods	Two-ker-nelled pods	Good nuts	Total kernels	Grand totals
Local Mauritius	1930	3·27	1·46	1·49	2·906	14·064	17·00	31·09	24·29
	1931	2·814	5·708	2·816	2·306	8·352	10·974	19·74	22·33
	1932	3·722	3·962	1·894	3·252	11·148	14·35	25·44	23·26
	1933	3·904	2·344	1·618	2·054	12·708	14·78	27·36	22·75
Gudiyattam bunch	1930	3·222	3·846	1·922	1·358	6·304	7·11	13·492	16·068
	1931	4·618	5·622	1·578	1·62	3·614	5·214	8·912	17·06
	1932	3·16	2·294	1·648	2·76	8·76	11·20	19·92	18·10

Apart from the lack of uniform maturity, there is also another factor which affects considerably the free fatty acid and the oil content of the sample. In many districts, particularly in those where the bunch type of groundnut is grown, the cultivator harvests the crop from one to two weeks before maturity, as he is afraid of the pods germinating in the soil and his not being able to enter the fields after heavy rains. The produce from such early harvests is inferior in quality. From Table I it may be noticed that the shipments from Calicut are the worst and contain as much as five to eight per cent of free fatty acids. The shipments from Calicut are mainly the groundnuts from Pollachi area where almost every year the crop is harvested either too early, or it is found not practicable to dry the produce properly on account of the rains. The early harvested groundnuts are generally very damp and, if not dried rapidly and properly, are liable to fermentation which increases the free fatty acid content and reduces the quantity of oil. The problem can, however, be met by cultivating a variety of even shorter duration than the ordinary bunch. Attempts are being made to evolve such a variety.

VIII. Summary and conclusions

As the groundnut seed develops, the percentage of oil gradually increases except in the early stages immediately following blooming and the period just preceding maturity. There is, throughout the development of the seed, a gradual and uniform gain in oil content and reduction in the free fatty acid content. The moisture content of the seed diminishes and the shelling percentage increases as the seed develops.

The harvest of groundnut even one week before the kernels are fully ripe, increases the quantity of free fatty acid and reduces the oil content of kernels by about five per cent. It is suggested that one of the reasons for the poor quality of South Indian groundnuts is the practice of harvesting groundnuts before they are fully mature. This practice not only increases the proportion of immature kernels but also increases the period required for drying the produce, and thus increases the chances of deterioration of the produce through fermentation which would be accelerated when the produce is wet.

The authors are indebted to Mr. V. Ramanatha Ayyar, the Cotton Specialist, for affording facilities to grow at his station the material required for the present investigation.

REFERENCES

Eyre, J. V. (1931). *Bio-Chem. J.* **25**, 1902.
Sahasrabuddhe, D. L. and Kale, N. P. (1933). *Ind. J. Agric. Sci.* **3**, 57.

INHERITANCE OF CHARACTERS IN *SETARIA ITALICA* (BEAUV.), THE ITALIAN MILLET

PART VII.—PLANT PURPLE PIGMENTATION

BY

G. N. RANGASWAMI AYYANGAR, B.A., I.A.S.,

Millets Specialist, Agricultural Research Institute,

T. R. NARAYANAN, B.Sc. Ag.,

T. NARAYANA RAO, M.A.,

AND

P. SESHADRI SARMA, B.Sc.Ag.,

Assistants, Millets Breeding Station, Coimbatore

(Received for publication on 9th January 1935)

(With Plates XI-XIII)

There are many varieties of the Italian millet distinguishable by their grain colours. Amongst varieties with the same grain colour separation is possible through bristles and earhead shapes. But at a breeding station where the whole life of the plant is being watched, a further grouping of the plants in the pre-maturity stages is possible. Apart from plant and leaf habits, purple pigmentation is the most striking character in the varietal grouping.

Plants of the Italian millet are broadly divisible into those with, and those without, purple pigmentation. A plant without purple pigmentation is called non-pigmented (No-P).

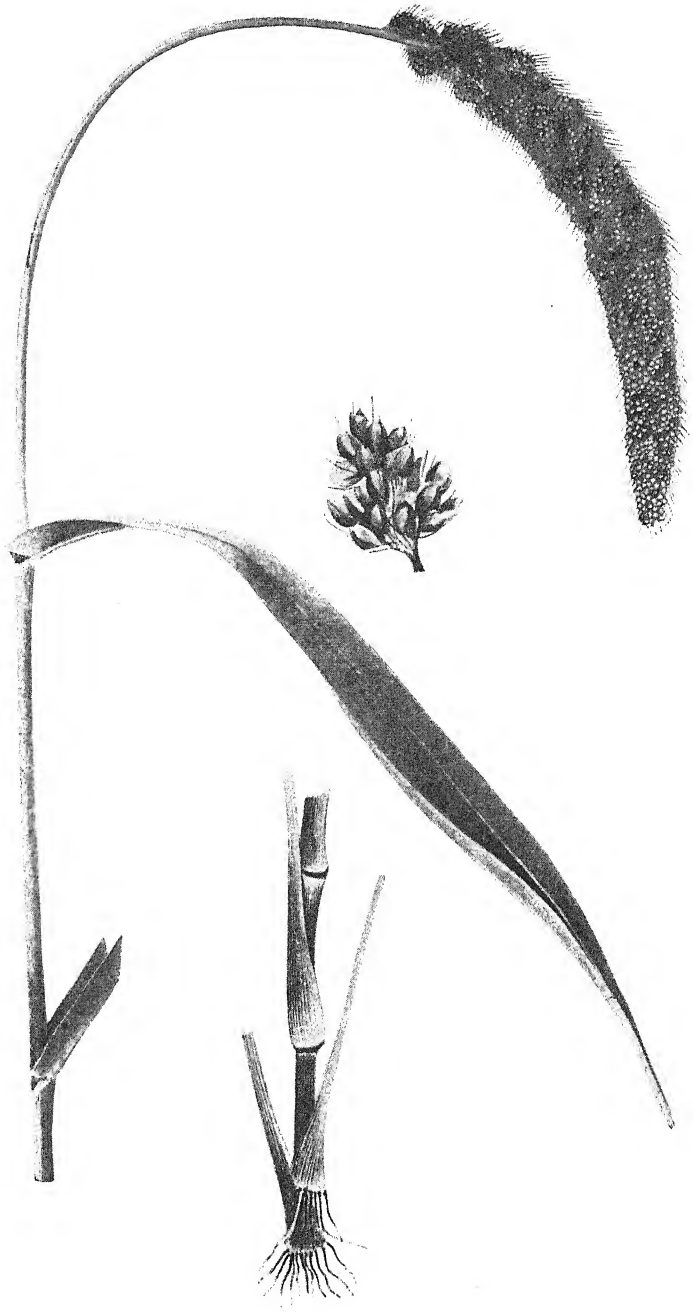
Plants with purple pigmentation fall into six classes according to the depth, distribution and mode of manifestation of the purple pigment on the plant. Seedlings give a general clue to the pigment classes. For this purpose seedlings of over 700 pure lines were examined and the development of pigmentation throughout the growing period for each of the various classes was observed during five seasons on 57 individual plants.

The lowest manifestation of purple pigmentation is in the last class designated P_6. The manifestation is so sparse that there is a risk of this being passed for a non-pigmented type. The seedlings of this P_6 are green. In the adult plant, the leaf-blade is occasionally coloured light purple. But the place to look for the

SETARIA ITALICA, (BEAUV.)

Pigment class—P_5

SETARIA ITALICA, (BEAUV.)



Pigment class—P_2

SETARIA ITALICA, (BEAUV.)

Pigment class—P_1

faint pigment is the earhead, a careful examination of which will show traces of purple pigment on the peduncle, on a few glumes and on stray bristle-tips. This pigmented class is best studied in the milky stage and the place to look for pigment is the edge of the glume. This class is characterised by light pigment in the earhead only and a late manifestation thereof.

The next class is P_5. In this class also the seedlings are green like P_6, and again the manifestation of the pigment is best at the flowering stage. The vegetative parts then take on a light purple colour and the earhead (the reproductive parts) appears a good purple. The light purple of the vegetative parts shows in the exposed roots, leaf-sheaths, leaf blades, nodes, and inter-nodes in a sparse manner and inconstantly. In the exposed roots, leaf-sheaths, leaf-blades and nodes, even this sparse colour can be detected only when the earhead ripens. This class is characterised by a dilute body pigment and a normal head pigment, both manifested late in the plant's life (Plate XIII).

P_6 and P_5 have this in common that the seedlings are green, and that the manifestation of purple pigmentation in both of them is late in the life of the plant.

The next four classes—P_4, P_3, P_2 and P_1—give indications of the presence of purple pigmentation in the seedling stage.

In P_4 the seedlings betray traces of light purple on their leaf-sheaths. In the adult plants the exposed roots and leaf-sheaths are light purple, the rest of the vegetative parts remaining green. In the earhead there is a sparse manifestation of light purple on odd glumes and bristle tips. This class is characterised by light purple seedlings. The manifestation of pigmentation is confined to some vegetative parts and is light in the reproductive parts.

The next class is the P_3. This class is a more steady and accentuated form of P_4. The seedling pigmentation is more easily recognised. The vegetative parts manifest light purple in all places, and the reproductive parts are also light purple. This class can be described as light purple throughout.

P_4 and P_3 have this in common, that the purple pigmentation is manifested lightly, but in P_4, the manifestation is restricted to some places only.

In the next two classes P_2 and P_1, the seedlings are distinctly purple. In the adults of P_1, there is a greater depth of purple in both the vegetative and the reproductive parts.

In the P_2 class the pigmentation is similar to the P_1 but there is a dilution on the reproductive parts, the pigmentation being sparse in the peduncles, glumes and bristles. The P_2 can be characterised as all purple with a dilution in the earhead (Plate XII).

The P_1 class is purple throughout and stands out as the best manifestation of purple pigmentation in the Italian millet (Plate XI).

Descriptions of the six types of pigment (P_1 to P_6) both as seedlings and as adults, are tabulated below :—

TABLE I

Seedlings

Pigment class	Exposed roots	Leaf sheaths	Leaf blades	Junction of leaf sheath and blade
P_1	Purple	P	P (stray)	P
P_2	Purple	P	P (stray)	P
P_3	Light purple	LP	LP (stray)	LP
P_4	Green	LP (stray)	G	G
P_5	Green	G	G	G
P_6	Green	G	G	G

TABLE II

Adults (vegetative parts)

Pigment class	Exposed roots	Leaf sheaths	Leaf blades	Junction of leaf sheath and blade	Nodes	Internodes
P_1	P	P	P (stray)	P	P	P
P_2	P	P	P (stray)	P	P	P
P_3	LP	LP	LP (stray)	LP	LP	LP
P_4	LP	LP (stray)	G	G	G	G
P_5	LP (stray)	LP (stray)	LP (stray)	G	LP (stray) when ripe	LP (stray) when ripe
P_6	G	G	LP (stray)	G	G	G

TABLE III
Adults (earheads)

Pigment class	Peduncles	Glumes	Bristles	Remarks
P_1	P	P	P	
P_2	P (stray)	LP (stray)	LP (stray)	Risk of passing for P_1.
P_3	LP (stray)	LP	LP	Risk of passing for P_1.
P_4	LP (stray)	LP (stray)	LP (stray)	Risk of passing for P_6.
P_5	P	P	P	Risk of passing for P_1 (when ripe).
P_6	LP (stray)	LP (stray)	LP (stray)	Risk of passing for No-P.

These six classes of pigmentation are distinct and with sufficient care and experience easily recognised. Pure lines of all the six types have been isolated and are breeding true.

The six pigmented classes differ obviously from the non-pigmented in having the basic factor (**P**) for pigmentation. P_1 and P_2 express clear purple pigmentation even in the seedling stages ; P_3 and P_4 also do so, but in a dilute degree. The seedlings of P_5 and P_6 are apparently green and develop pigmentation late in the life of the plant and that dilutely.

A factor (**I**) which determines the intensity with which pigmentation manifests itself, is present in P_1, P_2 and P_5, and absent in P_3, P_4, and P_6, and accounts for differential depths of manifestation of the purple pigment.

Purple pigmentation, whether intense or dilute, is also localised. Such pigment may be present in all the parts at which it could be present, or be localised in either the vegetative or earhead parts. A factor **V** determines clear manifestation in vegetative parts and a factor **H**, clear manifestation in the earhead. When both **V** and **H** are present, the pigmentation is general in distribution. **V** determining expression in vegetative parts, its presence shows right from the seedling stage and helps to mark off the seedlings of P_1, P_2, P_3 and P_4 from those of P_5 and P_6.

The interactions of **P, I, V** and **H** factors produce the diversity of forms characterizing varieties of this millet*. The nature and constitution of the six pigmented and the non-pigmented classes are given below :—

P_1—Has all the four factors and is a pigmented type of the highest degree (**PPVVHHII**).

P_2—Lacks the **H** factor, **V** and **I** being present. The absence of **H** results in a sparse and inconstant distribution of the pigment in the earhead

*Recent work on ' Genetics of Millets in India ', by G. N. Rangaswami Ayyangar, *Madras Agric. J.*, Vol. XXII, No. 1, P. 23. Paper contributed to the Symposium on ' Recent Work on Plant Genetics in India ', at the Indian Science Congress, Bombay, January 1934, Dr. P. S. Hudson, Deputy Director, Imperial Bureau of Plant Genetics, Cambridge, opening the discussion.

giving the head an almost greenish look when emerging (**PPVVhhII**).

P_3—Is like P_1 in distribution, but lacking the **I** factor, does not manifest the pigmentation in intensity. It is a P_1 dilute (**PPVVHHii**).

P_4—Has the **P** and **V** factors only; lacks **I** and **H**. The resultant type is practically a green earhead on a dilute purple pigmented vegetation (**PPVVhhii**).

P_5—Has **P, H** and **I** factors. The factor **V** is absent. This produces a good purple pigmented earhead, on a practically green vegetation (**PPvvHHII**).

P_6—Is the type with the sparsest pigment. Its earheads show traces of purple. Being an extreme recessive its genetic constitution will vary according to which class it is allelomorphic, but and resultant expression of extreme sparseness in pigmentation is constant. (**PPvvHHii**), or (**PPvvhhII**), or (**PPvvhhii**).

No-P—Varieties that are non-pigmented will have the following wide range of genetic constitutions, whose nature will not become evident until they are mated with pigmented types or have been extracted from pigmented allelomorphs. (**ppvvhhii, ppVVhhii, ppvvHHii, ppvvhhII, ppVVHHii, ppVVhhII, ppvvHHII,** or **ppVVHHII**).

A number of pure lines isolated and studied, and crosses purused at the Millets Breeding Station, Coimbatore, confirm the above analysis and are presented below :—

TABLE IV

Pure for **P, V, H** *and segregating for* **I**
Segregating for pigment classes P_1 *and* P_3

Season	Generation and family	Character of selection and constitution	Segregating for	
			P_1	P_3
1927	Natural cross			
1928	F_2 *S. I.* 758 . .	P_1 (**PPVVHHIi**) . .	57	20
1930	F_3 *S. I.* 1375 to 1379, 1382	P_1 (**PPVVHHII**) . .	Pure	..
	S. I. 1374, 1380, 1381	P_1	512	181
1931	F_4 (from *S. I.* 1374)			
	S. I. 1971, 1972 .	P_1	Pure	..
	S. I. 1970, 1973 to 1975.	P_1	402	136
	S. I. 1976, 1977 .	P_3 (**PPVVHHii**) . .	..	Pure
		Total .	971	337
		Expected .	981	327

$\chi^2 = 0{\cdot}41$ $P > {\cdot}5$

TABLE V

Pure for **P** *and* **V**, *segregating for* **H** *or* **I**

Segregating for pigment classes P_5 *and* P_6

Season	Generation and family	Character of selection and constitution	Segregating for P_5	Segregating for P_6
1927	Natural cross			
1928	F_2 *S. I.* 940 . .	P_5 (PPvvHhII) or (PPvvHHIi.	212	74
1930	F_3 *S. I.* 1521, 1522, 1525, 1530.	P_5 (PPvvHHII) . .	pure	..
	S. I. 1523, 1524, 1526, to 1529, 1531 to 1533.	P_5	785	23
	S. I. 1534 to 1538 .	P_6 (PPvvhhII) or (PPvvHHii).	..	pure
1931	F_4 *S. I.* 1990 . .	P_6	..	pure
		Total .	997	310
		Expected .	980	327

$\chi^2 = 1\ 14 \quad P > \cdot 2.$

Other experiences

1928	*S. I.* 941 clan.			
	S. I. 941 . . .	P_5	228	78
1929	*S. I.* 888 clan.			
	S. I. 1206, 1208 . .	P_5	400	122
	S. I. 1207, 1209 . .	P_5	pure	..
	S. I. 1210 . . .	P_6	..	pure
		Total .	628	200
		Expected .	621	207

$\chi^2 = \cdot 32 \quad P > \cdot 5$

TABLE VI

Pure for **P, V, I** *and segregating for* **H**

Segregating for pigment classes P_1 *and* P_2

Season	Generation and family	Character of selection and constitution	Segregating for	
			P_1	P_2
1931 (summer)	Artificial crosses . .	*S. I.* 1849 P_2 (PPVVhhII)		♀
	LXXV to LXXVII .	*S. I.* 381 P_1 (PPVVHHII)	♂	
1931 (rain-fed)	F_1	....	F_1	..
1932	F_2			
	S. I. 2264 to 2269 .	P_1 (PPVVHhII) . .	1033	369
		Total .	1033	369
		Expected .	1052	351

$\chi^2 = 1{\cdot}30$ $P > {\cdot}2$

Other experiences

1928	*S. I.* 942 *clan.*			
	S. I. 1248, 1823, 1825, 1828, 1830, 1832, 1845, 1847, 1848.	P_1	1539	518
	S. I. 1249, 1827, 1829, 1833, 1834, 1843, 1844, 1846.	P_1	Pure	..
	S. I. 1849, 1850 .	P_2	..	Pure
		Total .	1539	518
		Expected .	1543	514

$\chi^2 = {\cdot}04$ $P > {\cdot}8$

TABLE VII

Pure for **P, V, i,** *and segregating for* **H**

Segregating for pigment classes P_3 *and* P_4

Season	Generation and family	Character of selection and constitution	Segregating for	
			P_3	P_4
1925	Natural cross . .	....	..	..
1926	F_2 *S. I.* 269 . .	P_3 (PPVVHhii) . .	P_3 and P_4 (no counts).	
1928	F_3 *S. I.* 392, 393, 395 to 399, 401 to 404, 406, 407.	P_3 (PPVVHHii) . .	Pure	..
	S. I. 394, 400, 405, 408.	P_3 . . .	322	84
	S. I. 387, 388, 391 .	P_4 (PPVVhhii) .	..	Pure
1930	F^4 (from *S. I.* 405.)			
	S. I. 1317, 1321, 1322, 1325, 1329.	P_3	Pure	..
	S. I. 1318 to 1320, 1323, 1324, 1326, 1327.	P_3	448	140
	S. I. 1328 . .	P_4	..	Pure
1931	F_5 (from *S. I.* 1320).			
	S. I. 1966 . .	P_3	Pure	..
	S. I. 1962 to 1965, 1967.	P_3	229	90
	S. I. 1968, 1969 .	P_4	..	Pure
		Total .	999	314
		Expected .	985	328

$\chi^2 = 0{\cdot}81$ $P > {\cdot}3$

TABLE VIII

Pure for **P, H** *and* **I,** *segregating for* **V.**

Segregating for pigment classes P_1 *and* P_5

Season	Generation and family	Character of selection and constitution	Segregating for	
			P_1	P_5
1927	*S. I.* 296 *clan.*			
	S. I. 915, 916, 1224, 1226	P_1 (PPVvHHII) . .	814	271
1928	*S. I.* 756 *clan.*			
	S. I. 1094, 1095 .	P_1 . . .	318	116
1928	*S. I.* 942 *clan.*			
	S. I. 1824, 1826, 1831 .	P_1	618	174
		Total .	1750	561
		Expected .	1733	578

$\chi^2 = \cdot 66$ $P > \cdot 3$

TABLE IX

Pure for **P, H** *and* **i**, *and segregating for* **V.**

Segregating for pigment classes P_3 *and* P_6

Season	Generation and family	Character of selection and constitution	Segregating for	
			P_3	P_6
1922	Natural cross . .	....	..	..
1925	F_2 *S. I.* 85 . .	P_3 (PPVvHHii) . .	P_3 and	P_6 (no counts)
1926	F_3 *S. I.* 204. . .	P_3 (PPVVHHii) . .	Pure	..
	S. I. 203, 208 . .	P_3	384	85
	S. I. 205 to 207 .	P_6 (PPvvHHii) . .	..	Pure
1928	F_4 (from *S. I.* 203).			
	S. I. 323 . .	P_3	Pure	..
	S. *I.* 324 to 338 .	P_3	1477	536
	S. I. 339 to 354 .	P_6	..	Pure
	F_4 (from *S. I.* 208) . *S. I.* 364 . .	P_6	..	Pure
1929	F_5 (from *S. I.* 326) .			
	S. I. 1053, 1055 .	P_3	Pure	..
	S. I. 1054 . .	P_3	174	58
	S. I. 1056 . .	P_6	..	Pure
		Total .	2035	679
		Expected .	2036	679

$\chi^2 = \cdot 0005$ $P > \cdot 98$

TABLE IX—*contd.*

Season	Generation and family	Character of selection and constitution	Segregating for P_3	Segregating for P_6
	Other experiences			
1927	*S. I.* 742 *clan.*			
	S. I. 742, 993 to 995, 997, 999, 1001 to 1004.	P_3	1465	478
	S. I. 996, 998, 1000	P_3	Pure	..
	S. I. 1005 to 1008	P_6	..	Pure
1928	*S. I.* 746 *clan.*			
	S. I. 746, 1074 to 1078, 1741, 1742.	P_3	1222	394
	S. I. 1079, 1743, 1744	P_3	Pure	..
	S. I. 1080 to 1082, 1745	P_6	..	Pure
1929	*S. I.* 1035 *clan.*			
	S. I. 1035	P_3	151	43
1930	*S. I.* 878 *clan.*			
	S. I. 878	P_3	123	43
		Total	2961	958
		Expected	2939	980

$\chi^2 = \cdot 61$ $P > \cdot 3$

In the six tables given above (Tables IV to IX) monofactorial segregations between two pigment classes are presented. In the five following tables (Tables X to XIV) are given segregations involving the pigment classes and their pigmentless allelomorphs.

TABLE X

Segregating for **P** *and pure for* **V, H** *and* **I**

Season	Generation and family	Character of selection and constitution	Segregating for	
			P_1	No-P
1928 (summer)	Artificial crosses . .	*S. I.* 181 **No-P** (**ppVVHHII**).		♀
	I to IV . . .	*S. I.* 224 P_1 (**PPVVHHII**)	♂	
1928 (rain-fed).	F_1	....	F_1	
1929	F_2 *S. I.* 1020 to 1029 .	P_1 (**PpHHVVII**) . .	1781	606
1930	F_3 (from *S. I.* 1025) .			
	S. I. 1646, 1650, 1651, 1667, 1668.	P_1	Pure	..
	S. I. 1645, 1647 to 1649, 1652 to 1654, 1657 to 1662, 1665, 1666, 1669, 1670.	P_1	1236	445
	S. I. 1655, 1656, 1663, 1664.	**No-P**		Pure
	F_3 (from *S. I.* 1026).			
	S. I. 1677 . .	P_1.	Pure	..
	S. I. 1671 to 1676, 1678 to 1681.	P_1	531	210
	S. I. 1682 to 1690 .	**No-P**	..	Pure
	F_3 (from *S. I.* 1027).			
	S. I. 1707 . .	P_1	Pure	..
	S. I. 1697 to 1706 .	P_1	639	231
	S. I. 1691 to 1696, 1708 to 1712.	**No-P**.	..	Pure
1931	F_4 (from *S. I.* 1645) *S. I.* 1997, 2001 .	P_1	Pure	..
	S. I. 1998 to 2000, 2002.	P_1	373	123

TABLE X—*contd.*

Segregating for **P** *and pure for* **V, H** *and* **I**

Season	Generation and family	Character of selection and constitution	Segregating for	
			P_1	No-P
1931	F_4 (from *S. I.* 1647)			
	S. I. 2005 . .	P_1	Pure	..
	S. I. 2003, 2004, 2006	P_1	213	83
	F_4 (from *S. I.* 1659)			
	S. I. 2032, 2033, 2036, 2042.	P_1	Pure	..
	S. I. 2031, 2034, 2035, 2037 to 2041, 2043.	P_1	743	195
		Total .	5516	1893
		Expected .	5557	1852
	$\chi^2=1{\cdot}16$	$P>{\cdot}2$		
	Other experiences			
1925	*S. I.* 76 *clan*. . .	....	..	..
	S. I. 199, 200, 317 .	P_1	719	216
	S. I. 198, 318, 319 .	P_1	Pure	..
	S. I. 201, 320 . .	No-P.	..	Pure
1925	*S. I.* 121 *clan*. . .	....	..	..
	S. I. 121, 223, 225 .	P_1	413	147
	S. I. 222, 224 .	P_1	Pure	..
	S. I. 226, 227 . .	No-P	..	Pure
1925	*S. I.* 164 *clan* . .	....	..	..
	S. I. 164, 262, 263, 265	P_1	726	250
	S. I. 260, 261, 264 .	P_1 . . .	Pure	..
	S. I. 266, 267, 268 .	No-P	..	Pure

TABLE X—*concld.*

Segregating for **P** *and pure for* **V, H** *and* **I**

Season	Generation and family	Character of selection and constitution	Segregating for	
			P_1	No-P
1928	*S. I.* 754 *clan* . .	....	..	..
	S. I. 754 . . .	P_1	54	18
1929	*S. I.* 1938 *clan.* . .	....	..	..
	S. I. 1938, 2411, 2413, 2414.	P_1	274	88
	S. I. 2412 . . .	P_1	Pure	..
	S. I. 2415 . . .	No-P	..	Pure
932	F_2 (from artificial cross)			
	S. I. 2302 to 2307 .	P_1	692	281
	S. I. 2320 to 2324 .	P_1	213	58
	S. I. 2353 to 2358 .	P_1	324	108
	S I. 2411 to 2415 .	P_1	139	52
		Total .	3554	1218
		Expected .	3579	1193

$\chi^2=2{\cdot}69$ $P>{\cdot}3$

TABLE XI

Segregating for **P** *and pure for* **V, h** *and* **I**

Season	Generation and family	Character of selection and constitution	Segregating for	
			P_2	No-P
1928	*S. I.* 942 *clan.* . .	....	..	..
	S. I. 1836, 1837, 1839, 1841, 2128, 2130, 2131.	P_2 (PpVVhhII) . .	693	204
	S. I. 1835, 1838, 1840, 2129.	P_2 (PPVVhhII) . .	Pure	..
	S. I. 2132, 2133 . .	No-P (ppVVhhII) . .	..	Pure
		Total .	693	204
		Expected .	673	224

$\chi^2=2{\cdot}44$ $P>{\cdot}1$

TABLE XII

Segregating for **P**, *an pure for* **V**, **H**, *and* **i**

Season	Generation and family	Character of selection and constitution	Segregating for	
			P_3	No-P
1926	Natural cross . .	....	..	..
1927	F_2 *S. I.* 707 . .	P_3 (PpVVHHii) . .	90	35
1928	F_3 *S. I.* 980 . .	P_3 (PPVVHHii) . .	Pure	..
	S. I. 981 . .	P_3	77	30
	S. I. 982 . .	P_3	115	35
	S. I. 983 . .	No-P (ppVVHHii) .	..	Pure
		Total .	282	100
		Expected .	287	96
	χ^2=·28	P>·5		

TABLE XIII.

Segregating for **P**, *and pure for* **H**, **I** *and* **v**

Season	Generation and family	Character of selection and constitution	Segregating for	
			P_5	No-P
1927	Natural cross . .	....	..	..
1928	F_2 *S. I.* 751 . . .	P_5 (PpvvHHII) . .	90	33
1929	F_3 *S. I.* 1369 . .	P_5 (PPvvHHII) . .	Pure	..
	S. I. 1370 . .	P_5	174	56
	S. I. 1371 . .	P_5	91	32
	S. I. 1372 . .	P_5	81	30
	S. I. 1373 . .	No-P (ppvvHHII) .	..	Pure
		Total .	436	151
		Expected .	440	147
	χ^2=·16	P>·		
	Other experiences.			
1932	*S. I.* 2187 *clan* . .	....	..	..
	S. I. 2449, 2450, 2452	P_5	175	58

TABLE XIV

Segregating for **P**, *and pure for* **v**

Season	Generation and family	Character of selection and constitution	Segregating for	
			P_6	No-P
1927	Natural cross . .	....	..	..
1928	F_2 *S. I.* 747 . .	P_6 (PpvvHHii), (PpvvhhII) or (Ppvvhhii).	69	32
1929	F_3 *S. I.* 1084, 1086 .	P_6 (PPvvHHii), (PPvvhhII) or (PPvvhhii).	Pure	..
	S. I. 1083, 1085, 1087	P_6	587	196
1930	F_4 (from *S. I.* 1083) .			
	S. I. 1748, 1749 .	P_6	Pure	..
	S. I. 1746, 1747 .	P_6	350	86
		Total .	1006	314
		Expected .	990	330

$\chi^2=1{\cdot}03$ $P>{\cdot}3$

In the following two tables (Tables XV and XVI) segregation for **P** along with segregation for pigment classes among the purples, giving a 9 : 3 : 4 ratio are presented.

TABLE XV

Segregating for **P** *and* **H**, *and pure for* **V** *and* **I**

Season	Generation and family	Character of selection and constitution	Segregating for		
			P_1	P_2	No-P
1931 (summer)	Artificial crosses .	*S. I.* 1750 No-P (ppVVHHII) . .			♀
	LXXXI—LXXXIV .	*S. I.* 1849 P_2(PPVVhhII)		♂	
1931 (rain-fed)	F_1	P_1 (PpVVHhII) . .	F_1		
1932	F_2 *S. I.* 2271 to 2278 .	P_1	758	257	297
		Total .	758	257	297
		Expected (9 : 3 : 4) .	738	246	328
	$\chi^2=3{\cdot}96$	$P>{\cdot}1$			
	Other experiences				
1932	F_2 *S. I.* 2350 to 2352 . (from artificial cross)	P_1	76	24	39

TABLE XVI

Segregating for **P** *and* **V** *and pure for* **H** *and* **I**

Season	Generation and family	Character of selection and constitution	Segregating for		
			P_1	P_5	No-P
1929	Artificial crosses . .	*S. I.* 316 **No-P** (**ppVVHHII**).			♀
	XLIX—LII . .	*S. I.* 301 P_5 (**PPvvHHII**).		♂	
1930	F_1	....	F_1		
1931	F_2 *S. I.* 1925 to 1929 .	P_1 (**PpVvHHII**) . .	145	47	62
1932	F_3 (from *S. I.* 1929) .				
	S. I. 2394, 2401 .	P_1	50	12	30
		Total .	195	59	92
		Expected (9 : 3 : 4) .	195	65	87
	$\chi^2 = \cdot 84$	$P > \cdot 5$			
	S. I. 2395, 2397 . .	P_1 (**PPVvHHII**) .	P_1	P_5	
	S. I. 2392, 2393 . .	P_1 (**PpVVHHII**) .	P_1		**No-P**
	S. I. 2399 . . .	P_5		Pure	
	S. I. 2396, 2400 . .	P_5 (**PpvvHHII**) .		P_5	**No-P**
	S. I. 2398, 2402, 2403 .	**No-P** (**ppVVHHII**) or (**ppvvHHII**)			Pure
	Other experiences				
1932	*S. I.* 2187 *clan* *S. I.* 2443, 2444, 2447.	P_1	218	69	95
1932	F_2 *S. I.* 2296 to 2301, 2308 to 2313. (from artificial cross)	P_1	1024	369	494
		Total .	1242	438	589
		Expected (9 : 3 : 4) .	1276	425	567
	$= 2 \cdot 23$	$P > 3$			

In the following table is presented the history of a clan pure for **P** and giving a bifactorial segregation for four pigment classes.

TABLE XVII

Pure for **P** *and* **I**, *and segregating for* **V** *and* **H**

Segregating for pigment classes P_1, P_2, P_5 *and* P_6

Season	Generation and family	Character of selection and constitution	Segregating for			
			P_1	P_2	P_5	P_6
1931 (summer)	Artificial crosses . XCVII—XCIX .	*S. I.* 1369 P_5 (PPvvHHII)			♀	
		S. I. 1849 P_2 (PPVVhhII)		♂		
1931 (rain-fed)	F_1 . . .	P_1 (PPVvHhII) .	F_1			
1932 (summer)	F_2 *S. I.* 2186 .	P_1 . . .	P_1	P_2	P_5	P_6 (no counts)
1932 (rain-fed)	F_2 *S. I.* 2290 to 2295	P_1 . . .	338	111	108	46
1932 (rain-fed)	F_3 (from *S. I.* 2186)					
	S. I. 2431, 2433 2442.	P_1 . . .	166	58	61	29
		Total .	504	169	169	75
		Expected (9 : 3 : 3 : 1)	516	172	172	57
	$\chi^2=6\cdot07$	$P>1$				
	S. I. 2429 .	P_1 (PPVVHHII) .	Pure			
	S. I. 2434, 2436	P_1 (PPVVHhII) .	P_1	P_2		
	S. I. 2430 .	P_2 . . .		Pure		
	S. I. 2435, 2437	P_2 (PPVvhhII) .		P_2		P_6
	S. I. 2439 .	P_5 (PPvvHHii)			Pure	
	S. I. 2432, 2438 2440.	P_5 (PPvvHhII) .			P_5	P_6
	S. I. 2441 .	P_6 (PPvvhhII) .				Pure

The data presented in the above tables, full and elaborate as they are, were sought to be further confirmed by the following artificial crosses, in every one case obtaining an F_1 plant of the expected genetic constitution.

TABLE XVIII

Artificial crosses

Cross number	Parents		F_1s
	♀	♂	
CCLXIX, CCLXX	*S. I.* 1990 P_6 (PPvvHHii) .	*S. I.* 2133 No-P (ppVVhhII)	P_1
CCCIX . .	*S. I.* 364 P_6 (PPvvHHii) .	*S. I.* 268 No-P (ppVVHHII)	P_1
CCCXII, CCCXIII	*S. I.* 268 No-P (ppVVHHII) .	*S. I.* 364 P_6 (PPvvHHii) .	P_1
CCCXVI . .	*S. I.* 364 P_6 (PPvvHHii) .	*S. I.* 2133 No-P (ppVVhhII)	P_1
CCCXXXI .	*S. I.* 1968 P_4 (PPVVhhii) .	*S. I.* 1750 No-P (ppVVHHII)	P_1
CCCXXXII .	*S. I.* 1750 No-P (ppVVHHII)	*S. I.* 1968 P_4 (PPVVhhii) .	P_1

SUMMARY

Plants of the Italian millet are either pigmented (anthocyanic) or without purple pigment (non-pigmented). The former condition is dominant and arises by the basic presence of a factor **P**. There are various manifestations and intensities in this pigmentation. A factor **I** determines a manifestation in intensity. This is dominant to a manifestation in a weaker depth. The degree to which **P** is operative, in addition to being greatly influenced by the presence of **I**, is conditioned by two other factors **V** and **H**, which determine the alacrity with which **P** manifests in the vegetative or earhead parts. The interactions of **P**, **I**, **V** and **H** factors produce the diversity of forms characterising varieties of this millet.

Data from over 420 families are presented in support of the above hypothesis. A number of artificial crosses furnish confirmatory evidence.

NATURE OF OXIDISING PHOTO-CATALYSTS IN SOIL

BY

FAZAL-UD-DIN, B.Sc.,

Bacteriological Assistant, Agricultural Research Institute, Lyallpur

(Received for publication on 27th September, 1934)

Introduction

It has been shown quite recently by Dhar, Bhatacharya and Biswas [1933] that nitrification in soil in the tropics is more of a photo-chemical than bacterial nature. The soil is believed to act as a catalytic agent.

The catalysts in soil can be either organic or inorganic or both. Many mineral substances such as titania, zinc oxide, sodium uranate, alumina, and silica have been found to act as photo-catalysts on the oxidation of ammonium compounds [Rao and Dhar, 1931].

Silica and alumina though present in large quantities in soil have but feeble action and cannot account for the total catalytic action of the soils. In view of the alleged importance of photo-nitrification in tropical soils [*loc. cit.*] further investigation of the problem was considered desirable.

Determination of catalytic power

Appropriate amount of the substance with fifty c. c. distilled water was put in 300-c. c. Pyrex glass conical flasks in duplicate. These were plugged with cotton wool and sterilized. Mono-ethyl-amine (33 per cent solution) and aniline (pure) which were used as the test* compounds, were separately added with a sterile pipette at the rate of 0·5 c. c. per flask. The flasks thus prepared were exposed to the sun. Nitrous nitrogen was estimated by Griess Ilosvay's method. In estimating nitrous nitrogen in aniline solution, it was found necessary to add about 0·5 c. c. glacial acetic acid along with the reagent. Since the method is extremely sensitive readings less than 0·4 in half inch cell (equal to 0·001 mgm. per 100 c.c. solution) have not been considered in this paper. In the case of organic catalysts sterilization was omitted to avoid decomposition, but to check against changes due to bacterial action if any, control flasks similarly charged and wrapped in black paper were kept side by side with the exposed flasks.

* Mono-ethyl-amine and aniline were used in preference to ammonium sulphate because the latter had been found to be oxidised very slowly. It is not yet possible to say why it is so, but work is being carried on which is expected to throw some light on this point.

Experimental

In order to determine the nature of the photo-catalysts in soil, the following experiments were performed :—

I.—Catalytic action of different soils

Six soils were taken for this study :

1. Lyallpur soil (Clay loam).
2. Lyallpur soil (Loam).
3. Bara soil (Sodium soil, Barren).
4. Jhansi soil (30 per cent clay).
5. Pusa soil (33 per cent calcium carbonate).
6. Leaf mold soil.

Five grams of soil was used as a catalyst. Dark controls were also prepared and kept along side the exposed flasks. The contents of the flasks were analysed after thirteen days. The results are shown in Table I.

TABLE I

Showing the catalytic powers of various soils

Serial No.	Catalysts	Mgm. nitrous nitrogen per 100 c.c. solution	
		Aniline	Mono-ethyl-amine
1	Lyallpur soil	0·211	0·194
2	Lyallpur soil	0·058	0·376
3	Bara soil	0·275	0·233
4	Jhansi soil	trace	0·143
5	Pusa soil	0·032	0·181
6	Leaf mold soil	0·583	0·389

Another set without sterilization was also prepared and kept in the incubator at 30°C. Nitrous nitrogen was estimated after thirteen days. Bacterial oxidation of both the compounds was negligible. This however does not mean that photo-nitrification is more vigorous than bacterial process under all conditions. Under artificial conditions the nitrifying organisms are highly specific as regards their food requirements. In Omliansky's solution nitrifying organisms are well known to oxidise about five mgs. of ammonium sulphate nitrogen per 100 c. c. solution in a fortnight when incubated at 30°C., while the photo-oxidation of ammonium sulphate in the presence of soil is *nil* [Sarkaria and Fazal-ud-din, 1933] in the same period.

In another experiment the contents of the flasks similarly prepared were analysed immediately and after an exposure of two hours at noon. Except in the leaf mold soil which contained 0·006 mgm. nitrous nitrogen per ten gram soil, the amount of nitrous nitrogen present initially was negligible. There was no change in the nitrous nitrogen content of the flasks after exposure in the case of mono-ethyl-amine but in aniline flasks three soils 1, 3, and 6 gave positive nitrous reaction, not more than 1/10th of that obtained in the previous experiment.

Since the change does not take place in the dark, the reaction may well be regarded as photo-chemical; the soils seem to act catalytically is clear from a consideration of results obtained after two hours exposure.

The following conclusions may be drawn from Table I :—

1. Catalytic power varies with different soils.
2. The oxidising power of soil catalysts is different towards different compounds.
3. The catalyst in soil may be either a single substance or mixture of substances.

Since there was no dark reaction in the controls, these were omitted when working with soil constitutents.

II. Catalytic power of the soil extract and that of the insoluble residue

Fifty grams of soil was occasionally shaken for six days with 400 c. c. of water. The soil was filtered through filter paper in a Buchner funnel, and residue on the filter paper washed four times with 25 c. c. of water. The washings were mixed with the extract, which was removed. The residue was further washed with distilled water till free from soluble material as judged by the absence of chlorides. The catalytic power possessed by 50 c. c. of the above extract (equivalent to 5 gms. original soil) and 5 gms. of the residue was determined after thirteen days' exposure.

The results given in Table II show that :—

(1) The catalyst is both water soluble and insoluble.

(2) The residue is a better catalyst for mono-ethyl-amine than aniline. With the extract it is quite the reverse.

TABLE II

Showing the catalytic powers of soil extract and residue (mgm. nitrous nitrogen per 100 c. c. solution)

Serial No.	Extract		Residue	
	Aniline	Mono-ethyl-amine	Aniline	Mono-ethyl-amine
1	0·178	0·039	0·032	0·272
2	0·060	0·042	0·032	0·194
3	0·227	0·071	0·031	0·168
4	trace	0·024	0·029	0·168
5	0·022	0·019	0·027	0·181
6	0·648	0·104	0·041	0·337

III. CATALYTIC POWER OF THE IGNITED SOIL

All the six soils were ignited at 600°C. for three hours in the muffle furnace and their catalytic power determined, using five grams of the soil as before. Controls without mono-ethyl-amine or aniline were also exposed simultaneously. The contents of the flasks were analysed after five days' exposure. The soils showed only feeble catalytic action.

It seems that the loss of catalytic power is mainly due to the destruction of organic matter and soil colloids. These were tested separately.

1. Organic matter

Concentrated soil extract was prepared from *bara* and leaf-mold soils by shaking these with an equal amount of distilled water, and then filtering. The extract in five c. c. portions was treated with varying amounts of Merck's hydrogen peroxide (12 Vol. 'O') in well corked test tubes and kept overnight. The contents of the tubes were then transferred with distilled water in the conical flasks and the volume made up to fifty c. c. The catalytic power of the extracts was determined as usual.

The results given in Table III show that the catalytic action of *bara* soil extract is not affected by treatment with hydrogen peroxide, whereas in the case of leaf-mold soil, it decreased with increasing amount of hydrogen peroxide.

TABLE III

Leaf mold soil extract		*Bara* soil extract	
Treated with	Mgm. nitrous nitrogen per 100 c.c. solution	Treated with	Mgm. nitrous nitrogen per 100 c.c. solution
2·0 c.c. hydrogen peroxide.	0·551	1·0 c.c. hydrogen peroxide.	0·251
4·0 c.c. hydrogen peroxide.	0·324	2·0 c.c. hydrogen peroxide.	0·251
6·0 c.c. hydrogen peroxide.	0·259	3·0 c.c. hydrogen peroxide.	0·251
8·0 c.c. hydrogen peroxide.	0·162	4·0 c.c. hydrogen peroxide.	0·251
Control . . .	0·972	Control .	0·243

It would seem that the soluble organic matter from *bara* soil is either not attacked by hydrogen peroxide or it possesses a very slight catalytic power.

The soil extracts corresponding to five grams soil from both the soils were evaporated and the residue ignited at a low temperature and its catalytic power was determined after ten days' exposure.

The results given in Table IV provide additional proof of the fact that the soil organic matter acts as a catalyst.

TABLE IV

Showing the catalytic power of the soil extract and its ash

Serial No.	Catalyst	Mgm. nitrous nitrogen per 100 c.c. solution
1	Extract of soil No. 6	0.236
2	Ash of the above	0.045
3	Extract of soil No. 3	0.178
4	Ash of the above	0.032
5	Control	0.016

2. *Soil colloids*

(*a*) It is well known that collodion membranes generally possess the power of retaining colloids. The catalytic power of soil extracts filtered through collodion membrane and ordinary filter paper was determined as usual. It was found to be unaffected by filtration through collodion membrane. This shows that the colloidal matter in the extract, if any, has no catalytic power. The results are given in Table V.

TABLE V

Showing the catalytic action of soil extract when filtered through collodion membrane and ordinary filter paper

(Mgm. nitrous nitrogen per 100 c.c. solution)

Catalyst	Extract No. 1		Extract No. 2
	Aniline	Mono-ethyl-amine	Aniline
Extract through collodion filter	0·713	0·123	0·113
Extract through filter paper .	0·713	0·117	0·113

(*b*) Clay fractions (0·002 mm.) were separated from the soil residues as prepared under experiment II. In order to get rid of organic matter*, the clays were treated with Merck's hydrogen peroxide for twenty-four hours, when these were filtered and washed with distilled water ; extracted with ether in the Soxhlet extraction apparatus for about eight hours ; and then further extracted with redistilled alcohol for three hours ; filtered and again washed with alcohol a number of times and dried.

The clays so prepared were taken in two-gram portions and tested for catalytic action against mono-ethyl-amine. Nitrous nitrogen was determined after thirteen days' exposure. The results given in Table VI show that the clay acts as a catalyst.

* It is surely desirable to completely remove organic matter from the clay, but this is not possible without more drastic treatment, which may also change the clay complex, hence treatment with hydrogen peroxide followed by extraction with ether and alcohol was considered sufficient.

TABLE VI

Showing the catalytic action of clays from different soils

Catalyst	Mgm. nitrous nitrogen per 100 c.c. solution
Clay from soil No. 1	0·110
Clay from soil No. 4	0·052
Clay from soil No. 5	0·065
Control	0·013

Having thus found that the catalysts in soil are both organic and inorganic in their nature more elaborated study of these two groups was made.

IV. NATURE OF THE ORGANIC CATALYSTS IN SOIL

1. Soil fats and waxes

One hundred grams of leaf-mold soil was extracted with 200 c.c. ether in the Soxhlet extraction apparatus for twelve hours. Ether extract corresponding to ten grams soil was measured out in each flask and evaporated to dryness over water bath. The catalytic power of the ether residue was determined in the usual way. To avoid decomposition of the organic matter the flasks were not sterilized and instead dark controls were kept side by side with the exposed flasks.

The results given in Table VII show that ether-soluble organic matter, *i.e.* soil fats and waxes possess a fair amount of catalytic power.

TABLE VII

Showing the catalytic action of ether extract

Treatment	Mgm. nitrous nitrogen per 100 c.c. solution	
	Aniline	Mono-ethyl-amine
1 Ether extract (exposed)	trace	0.078
2 Ether extract (unexposed)	*nil*	*nil*
3 Control (without catalyst)	*nil*	0.029

2. *Soil resins*

The soil after treatment with ether was extracted with absolute alcohol over water-bath for about four hours. The extract was filtered and portions corresponding to ten grams soil were measured out in flasks. These were evaporated to dryness over water-bath, and the catalytic power of the residue was determined as in the case of ether extract.

The results given in Table VIII show that soil resins also have catalytic action.

TABLE VIII

Showing the catalytic action of alcoholic extract

Treatment	Mgm. nitrous nitrogen per 100 c.c. solution	
	Aniline	Mono-ethyl-amine
Alcoholic extract (exposed)	0·810	0·047
Alcoholic extract (unexposed)	*nil*	*nil*
Control (without catalyst)	*nil*	0·029

The mineral matter in the extract was also found to be slightly catalytic in action.

3. *Humus.*

Humus was extracted from soil in the usual way. It was purified by redissolving and precipitating. Finally the humus or to be more correct humic acid precipitated with hydrochloric acid was washed with distilled water a number of times to get rid of ammonium chloride and hydrochloric acid. It was shaken with distilled water in a stoppered cylinder and a portion corresponding to five grams soil was tested as a catalyst. The catalytic power was determined in the usual manner. The analysis was done after twelve days' exposure.

The results given in Table IX show that both humus and its ash act as catalysts.

TABLE IX

Showing the catalytic action of humus

Serial No.	Treatment	Mgm. nitrous nitrogen per 100 c.c. solution
1	Humus with mono-ethyl-amine (exposed) . .	0·130
2	Humus with mono-ethyl-amine (unexposed) .	*nil*
3	Humus ash with mono-ethyl-amine (exposed) .	0·071
4	Humus without mono-ethyl-amine (exposed) .	*nil*
5	Control	0·026

4. Cellulose

The catalytic power of Whatman filter paper cellulose was determined using one gram filter paper as a catalyst.

The results given in Table X show that impure cellulose acts as a catalyst.

TABLE X

Showing the catalytic action of filter paper

Catalyst	Mgm. nitrous nitrogen per 100 c.c. solution	
	Aniline	Mono-ethyl-amine
Filter paper	Trace	0·042
Control	*Nil*	0·016

The experiment was repeated with purified cellulose from cotton wool. The results were negative.

The catalytic power of impure cellulose was found to have increased after bacterial decomposition.

Fifty c. c. culture medium of the composition given below* was transferred to each of the four 300-c. c. conical flasks. One gram filter paper strips were added to each.

These were inoculated with a drop of fresh horse dung extract. Two of these were sterilized, and kept along with the other two in the incubator at 30°C. for a month. When the cellulose in the unsterilized flasks was macerated, the catalytic power of the contents of the flasks against mono-ethyl-amine was determined after six days' exposure. The catalytic action of the decomposed filter paper was found to have increased. The results are given in Table XI.

Potassium hydrogen phosphate	1·0 gram.
Magnesium sulphate	1·0 ,,
Sodium chloride	1·0 ,,
Ammonium sulphate	2·0 ,,
Distilled water	1000 c.c.

TABLE XI

Showing the catalytic action of filter paper and its decomposition products

Serial No.	Catalyst	Mgm. nitrous nitrogen per 100 c.c. solution
1	Decomposed filter paper	0·062
2	Filter paper	0·036
3	Control	0·014

Cellulose present in soil is very likely to behave as filter paper above. The increased action may be due either to the decomposition products of cellulose or to the bacterial bodies. This point is still to be investigated. There is a certain amount of evidence to show that bacterial bodies also act as photo-catalysts. The work is in progress.

Since soil organic matter is mostly derived from plant waste materials, extract from green plants was also tried as a catalyst. A few green stalks of berseem (*Trifolium alexandrinum*) well washed with distilled water were extracted with absolute alcohol. Five c. c. of the extract was evaporated to dryness over water-bath in a flask and the catalytic power of the residue was determined without sterilizing but with dark controls. The contents of the flasks were analysed after four days'

exposure. The alcoholic extract of the green stalks exhibited catalytic property. Extract of non-legumes also showed a similar action. Results are given in Table XII.

TABLE XII

Showing the catalytic action of alcoholic extract of green berseem leaves

Treatment	Mgm. nitrous nitrogen per 100 c.c. solution	
	One per cent mono-ethyl-amine	*N*/10 ammonia
Alcoholic extract (exposed)	0·097	0·014
Alcoholic extract (unexposed)	*nil*	*nil*
Control (exposed)	0·012	Trace

Aquous extracts of the plants were also found to be catalytic in action.

This shows that plant leaves have the power of oxidising ammonia in sun light. Free ammonia is said to be toxic to plant life, but the plant may be able to utilize it because of its catalytic power. Work on plant catalysts is in progress.

5. Inorganic catalysts in soil

Since soil is mainly a mineral complex, the method of study adopted in this case was to try different metallic bases as catalysts.

In most cases the oxides of the metals were used. The catalytic power possessed by one gram of the bases was determined as before using aniline and mono-ethyl-amine as the 'substrate'. Exposed and unexposed flasks were kept both, with and without the 'substrate'.

Since the oxides are generally prepared by the ignition of nitrates there is possibility of their contamination with nitrates, nitrites or other nitrifiable compounds. So in order to avoid errors due to these sources, controls (both exposed and unexposed) without the 'substrate' were also prepared.

The contents of the flasks were analysed after a fortnight. Oxides of lithium, manganese, bismuth, lead, nickel, copper, mercury, and chlorides of potassium calcium, barium, magnesium, showed catalytic action. Stannic chloride, antimony, tetroxide and ferric oxide gave negative results. The action of the remaining compounds, with the exception of bismuth trioxide and mercuric oxide was very feeble

This would indicate that the catalytic action of the soil is mainly a function of the organic matter it contains.

It has been found that the nature of the acid radical in combination with the base considerably modifies the catalytic power of the metallic ion; the catalytic properties of some of the more important clays were also investigated.

A clay soil was taken, and it was exhaustively washed with $N/20$ hydrochloric acid till the filtrate gave no test for calcium. It was now washed free of acid with distilled water. The clay (0·002 mm.) was separated by centrifuging. It was dried over water-bath, treated with Merck's hydrogen peroxide over night, again dried, extracted with ether and then with alcohol. Different clays were prepared from this by washing the hydrogen clay over Buchner funnel with small quantities of normal solutions of the metallic clorides. The washings were continued till the filtrate gave no acid reaction. The clay was then washed with distilled water till free from chlorides.

Two grams of the clay as prepared above was used as a catalyst for the oxidation of mono-ethyl-amine. Dark controls were also kept. Nitrous nitrogen was determined after sixteen days' exposure. The results given in Table XIII show that the different clays are catalytic in action and sodium and potassium clays are more vigorous in this respect than the others.

TABLE XIII

Showing the catalytic action of different clays

Serial No.	Catalyst	Mgm. nitrous nitrogen per 100 c.c. solution
1	Control	0·029
2	Calcium clay	0·039
3	Hydrogen clay	0·060
4	Magnesium clay	0·065
5	Manganese clay	0·036
6	Potassium clay	0·110
7	Sodium clay	0·110

Conclusions

1. Catalytic power varies with different soils.

2. The oxidising power of a catalyst differs with different compounds.

3. Soil fats, waxes, resins and humus act as catalysts.

4. Impure cellulose possesses catalytic power, which increases on its bacterial decomposition.

5. Alcoholic extract as well as aquous extract of plants also show catalytic action.

6. Water soluble mineral matter in the soil, clay, chlorides of potassium, barium, calcium, magnesium, and oxides of manganese, lithium, bismuth, lead, nickel, copper and mercury all possess some catalytic power which is quite well pronounced in the case of bismuth and mercury.

7. Sodium and potassium clays are better catalysts than calcium, hydrogen, magnesium and manganese clays.

8. The bacterial nitrification in mono-ethyl-amine and aniline solutions was negligible as compared to the photo-nitrification in the presence of soil.

REFERENCES

Dhar, N. R. ; Bhatacharya, A. K. and Biswas, N. N. (1933). *Soil Sci.* **35**, 281.
Sarkaria, Ram Singh and Fazal-ud-din (1933). *Ind. J. Agric. Sci.* **3**, 1057.
Rao, G. G. and Dhar, N. R. (1931). *Soil Sci.* **31**, 379.

PHOTO-OXIDATION OF SULPHUR

BY

FAZAL-UD-DIN, B.Sc.,

Bacteriological Assistant, Agricultural Research Institute, Lyallpur

(Received for publication on 14th January, 1935)

INTRODUCTION

Bacterial oxidation of elemental sulphur and its chemical oxidation at a high temperature are well known. Kappen and Quensell [1915] were the first to show that chemical oxidation of sulphur at room temperature is possible under the influence of soil catalysts and that considerably large quantities of sulphur are oxidised in unsterile than in sterile soils. These findings were later confirmed by MacIntire, Gray and Shaw [1921].

While photo-oxidation of certain nitrogenous compounds was being studied in this laboratory, it seemed interesting to see if elemental sulphur could also be oxidised photo-chemically.

Experimental

One gram of Kahlbaum's precipitated sulphur and fifty c. c. of water were taken in each of thirty-two Pyrex glass flasks of 300 c. c. capacity. These were divided into four sets and each set was submitted to the treatment as shown below :—

1. No treatment.
2. One gram zinc oxide per flask.
3. One gram animal chracoal per flask.
4. Five grams field soil per flask.

A fifth set of eight flasks each containing fifty c. c. of distilled water and five grams of soil was also prepared to see if there was any change in the sulphate content of the soil on exposure to the sun.

The flasks were plugged with cotton wool and sterilized in flowing steam for thirty minutes on three consecutive days and the plugs covered with paper to protect them from dust. Four flasks in each set were wrapped with black paper and these along with the rest were kept in the sun.*

The contents of the flasks were analysed for sulphates by the usual gravimetric-method after sixty days and the results are given in Table I.

* The experiments described in this paper were all carried out during summer months.

TABLE I

Showing photo-oxidation of sulphur

Set No.	Description	Grm. barium sulphate per flask		Mean difference
		Exposed	Unexposed	
1	Sulphur without catalyst .	0·0044	Trace	0·0050
		0·0042	,,	
		0·0062	,,	
		0·0054	,,	
2	Sulphur + zinc oxide . . .	0·0124	Trace	0·0097
		0·0098	,,	
		0·0090	,,	
		0·0078	,,	
3	Sulphur + animal charcoal . . .	0·0964	0·0500	0·0443
		0·0926	0·0430	
		0·0900	0·0478	
		0·0888	0·0496	
4	Sulphur + soil . . .	0·0700	0·0164	0·0534
		0·0726	0·0172	
		0·0684	0·0162	
		0·0670	0·0148	
5	Soil without sulphur . .	0·0078	0·0078	0·0001
		0·0072	0·0070	
		0·0072	0·0074	
		0·0076	0·0072	

The original sulphates in one gram of animal charcoal and five grams of soil were also estimated and found to be 0·0100 grm. and 0·0074 grm. respectively when weighed as barium sulphate.

It is evident from the above figures that sulphur is oxidised photo-chemically. Zinc oxide showed only a feeble catalytic action. The action of animal charcoal and soil, however, was more pronounced.

In the cases of animal charcoal and soil the oxidation of sulphur proceeded also in the dark.

Another experiment was conducted to see if the oxidation of sulphur could be stimulated by an alkaline solution.

One gram of sulphur, two grams of animal charcoal and fifty c.c. of water were added to eight different flasks. Of these four flasks received 5·0 c.c. $N/10$ sodium hydroxide each and the flasks after sterilization were exposed to the sun. The contents of the flasks were analysed after five months. The results given in Table II show a slightly greater degree of oxidation in the flasks containing soda.

TABLE II

Showing the oxidation of sulphur in the presence and absence of sodium hydroxide

Serial No.	Gm. barium sulphate per flask		Difference
	With sodium hydroxide	Without sodium hydroxide	
1	0·1770	0·1722	0·0048
2	0·1698	0·1582	0·0116
3	0·1776	0·1600	0·0176
4	0·1734	0·1558	0·0176
Mean	0·1744	0·1615	0·0129

A statistical examination of the differences given in the last column of the above table was made. These values were found to be subject to a standard deviation of $\pm 0{\cdot}0061$. The standard error of the mean difference was therefore estimated to be $\frac{0{\cdot}0061}{\sqrt{4}}$ or 0·003. The significance of the mean difference 0·0129 was tested against its standard error by Fisher's 't'-test. The value of 't' was determined to be 4.2. The expected value of 't' from Fisher's 't'-table for level of significance 0·05, was found to be 3·18, which is less than the value obtained from the differences examined. The effect of caustic soda on the oxidation of sulphur was thus found to be quite significant.

The oxidation of sulphur in soil may either be bacterial, chemical or photochemical. In order to compare the bacterial oxidation of sulphur in soil with the other two processes, 200 grms. of soil were taken in three separate conical flasks of 500 c. c. capacity and two per cent of sulphur was added in two of them. A moisture* content of 13 per cent was supplied to all three. In one of the flasks containing sulphur moisture was supplied by the aid of a mercuric chloride solution saturated

*Thirteen per cent moisture was the optimum for the activity of aerobic bacteria in the soil.

at 25°C. and in the others with distilled water. The flasks were exposed to the sun, and the loss of moisture was made up from time to time, and the contents of the flasks analysed after sixty days. The absence of sulphur-oxidising bacteria in soil treated with mercuric chloride was tested before analysis. About a gram of the soil was inoculated in each of the six conical flasks containing fifty c.c. of sterile medium of the following composition :—

Ammonium sulphate	0·2 grm.
Magnesium sulphate	0·5 „
Mono-potassium phosphate	1·0 „
Calcium phosphate reprecipitated	2·5 grms.
Sulphur	10·0 „
Distilled water	1000 c.c.

$\frac{M}{1}$ phosphoric acid suffientie to adjust the reaction to pH 4·0.

Three of the flasks were again sterilized and these along with the rest were kept in the incubator at 30°C. The reaction (pH) of the contents in each flask was determined after six weeks and was found to be the same in all cases, showing that the soil was free from sulphur-oxidising bacteria.

The results given in Table III show that chemical oxidation of sulphur in soil is very slight compared with biological oxidation.

TABLE III

Showing the oxidation of sulphur in soil

Treatment	Grm. barium sulphate from 5 grams of soil
Soil alone	0·0058
Soil + sulphur	0·2222
Soil+sulphur+mercuric chloride	0·0092

CONCLUSIONS

1. Elemental sulphur is oxidised photo-chemically.

2. Zinc oxide, animal charcoal and soil act as photo catalysts.

3. Chemical oxidation of sulphur in soil is negligible compared with the biological effect.

REFERENCES

Kappen, H., and Quensell, E. (1915). Uber die Umwandlungen von Schwefel und Schwefelverbindungen im Ackerboden, ein Beitrag zur Kenntnis des Schwefel-Kreislaufes : *Landw. Vers. Stat.* **86**, 1—34.

MacIntire, W. H. ; Gray, F. J. ; and Shaw, W. M. (1921). *Soil Sci.* **11**, 249.

SUGARCANE IN THE PUNJAB, PART I

BY

P. E. LANDER, M.A., D.Sc., F.I.C.,

Agricultural Chemist to Government, Punjab

AND

RAMJI NARAIN, PH.D., D.Sc.,

Assistant to the Agricultural Chemist, Punjab, Lyallpur

(Received for publication on 2nd October 1934)

(With Plates XIV and XV and sixteen text-figures)

CONTENTS

PREFACE

The authors wish to record their appreciation and acknowledgment of the valuable assistance given by the Deputy Directors of Agriculture and their assistants of the Hansi, Jullundur and Gurdaspur Circles, in carrying out these surveys. They also wish to record the ready co-operation afforded by zamindars in all the areas mentioned, in reserving plots of cane for analysis and other valuable help.

The surveys described in this paper were carried out by Mr. K. K. Paracer, S. Balwant Singh Mahon, and L. Jiwan Dass, of the Chemical Section in the Karnal-Rohtak, Jullundur-Hoshiarpur and Gurdaspur-Sialkot districts, respectively. Our acknowledgments are due to them for the painstaking care and zeal with which they carried on this work. Special credit is due to S. Balwant Singh for his assistance in the enormous amount of work entailed in the computation and arrangement of the data which represent the results from over four thousands analyses.

Lyallpur,
15th August 1934

P. E. LANDER

RAMJI NARAIN

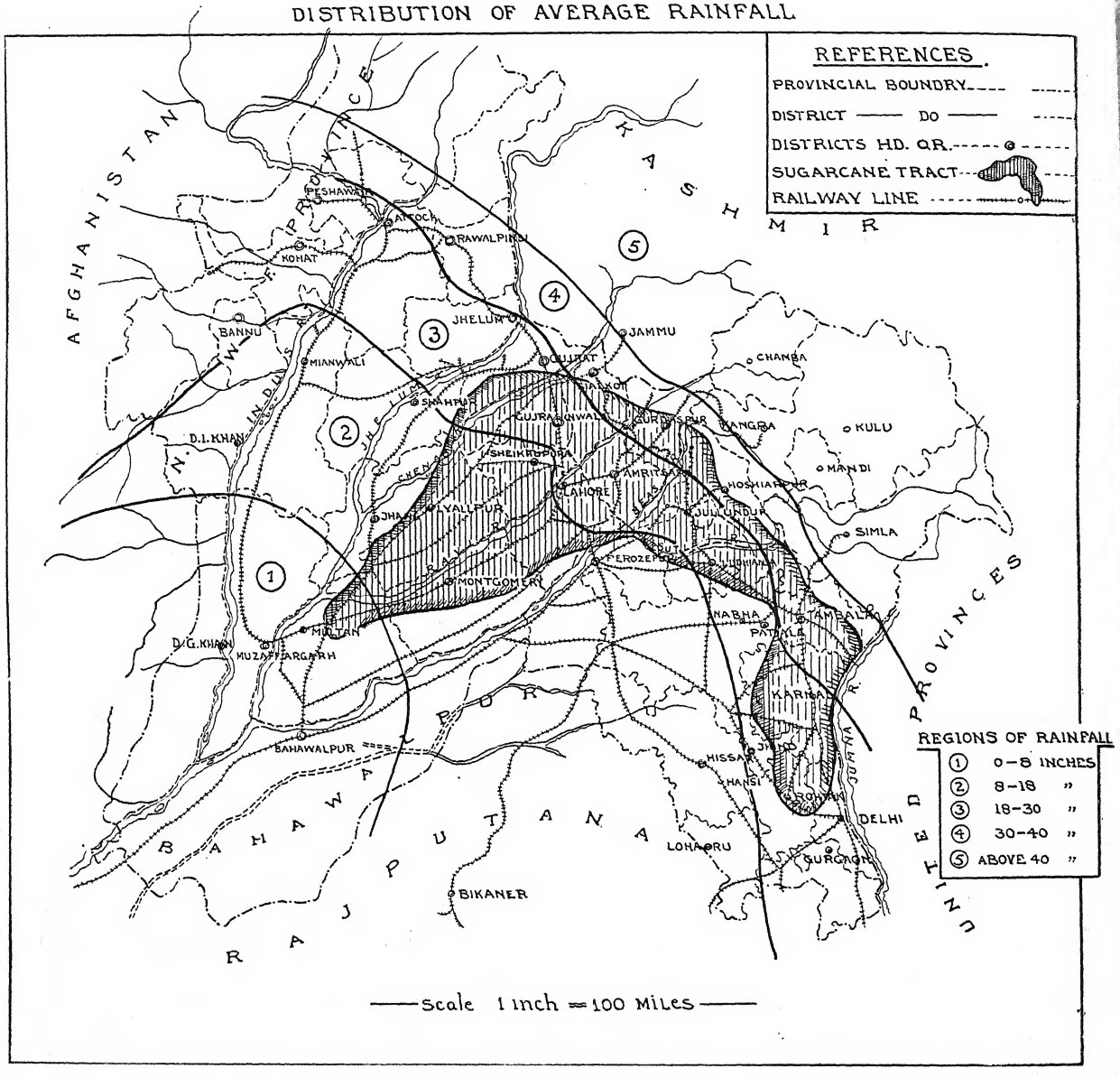
MAP OF THE PUNJAB

SHOWING.

MAIN SUGARCANE TRACT INCLUDING DISTRICTS WITH AN ACREAGE OF 10,000 OR ABOVE

&

DISTRIBUTION OF AVERAGE RAINFALL



INTRODUCTION

The activities of the Punjab Department of Agriculture in investigating and developing the production of cane in this Province date back to 1911, when Gurdaspur, the district with the then largest area under cane, was selected for starting work on varietal tests.

These tests were carried out at the Gurdaspur Agricultural Experimental Station over a period of seven years, and the results published as a Bulletin of Agricultural Research Institute, Pusa [Barnes, 1918].

Similar work was also carried out during this period at Lyallpur.

The necessity for more extended systematic work both on cane growing in the field, and factory requirements was recognised, and in 1919 the Government of India appointed the Indian Sugar Committee under the Chairmanship of Mr. J. Mackenna, Agricultural Advisor to the Government of India, whose terms of reference were to investigate the possibilities of organising and developing the sugar industry of India.

This Committee published its report in 1921 and made certain specific recommendations in regard to the Punjab, to the effect that work should be extended on trials of improved varieties imported from other Provinces, exotics, and also improved crosses from Coimbatore.

These recommendations were given effect to, and as a result of subsequent investigations on most of the main Government Farms, a number of approved new varieties was adopted by the cultivators of the Punjab.

In August 1926 Mr. Noel Deerr of Messrs. Begg, Sutherland & Co. was invited by the Director of Agriculture, to tour the principal cane growing areas of the Punjab with the object of studying the cane situation then existing, and to advise on the possibility of establishing efficient modern factories to supersede the acknowledged inefficient methods in vogue.

Mr. Noel Deerr recognised that some of the new varieties such as Co. 205, Co. 223, etc., had been definitely adopted in various parts of the Province and that theygave yields far in excess of those hitherto obtained by the old indigenous varieties such as *Katha, Kahu, Dhaulu*, etc. In his report submitted in November 1926, he also laid stress on the fact that for the successful working of a sugar factory it was essential that the crushing season be extended to the middle of April and that the high yielding new Coimbatore canes should supplant the *desi* varieties.

This report was considered by the Sugar Sub-Committee of the Joint Development Board, as a result of whose deliberations, it was suggested that the Agricultural Chemist to Government, Punjab, should carry out a systematic analytical survey

of the canes grown in the Karnal, Gurdaspur, and Jullundur districts, as it was considered that these were the districts which might prove most suitable for establishing factories.

An important aspect of this enquiry was to ascertain the effect of frost and other factors on the various varieties grown in these areas, with the particular object of finding out those canes which could keep a factory supplied with ripe raw material throughout an economic working season.

A systematic enquiry based on these lines was accordingly carried out in the districts mentioned over a series of years from 1927 to 1932, the results of which are presented in the following pages.

The following localities were surveyed :—

1. Karnal and Rohtak districts, representing the south-eastern sugarcane growing tract of the Province (the survey in this area came to an end in 1930-31).
2. Jullundur and Hoshiarpur districts, representing the Central Punjab and,
3. Gurdaspur and Sialkot districts representing the sub-montane tracts.

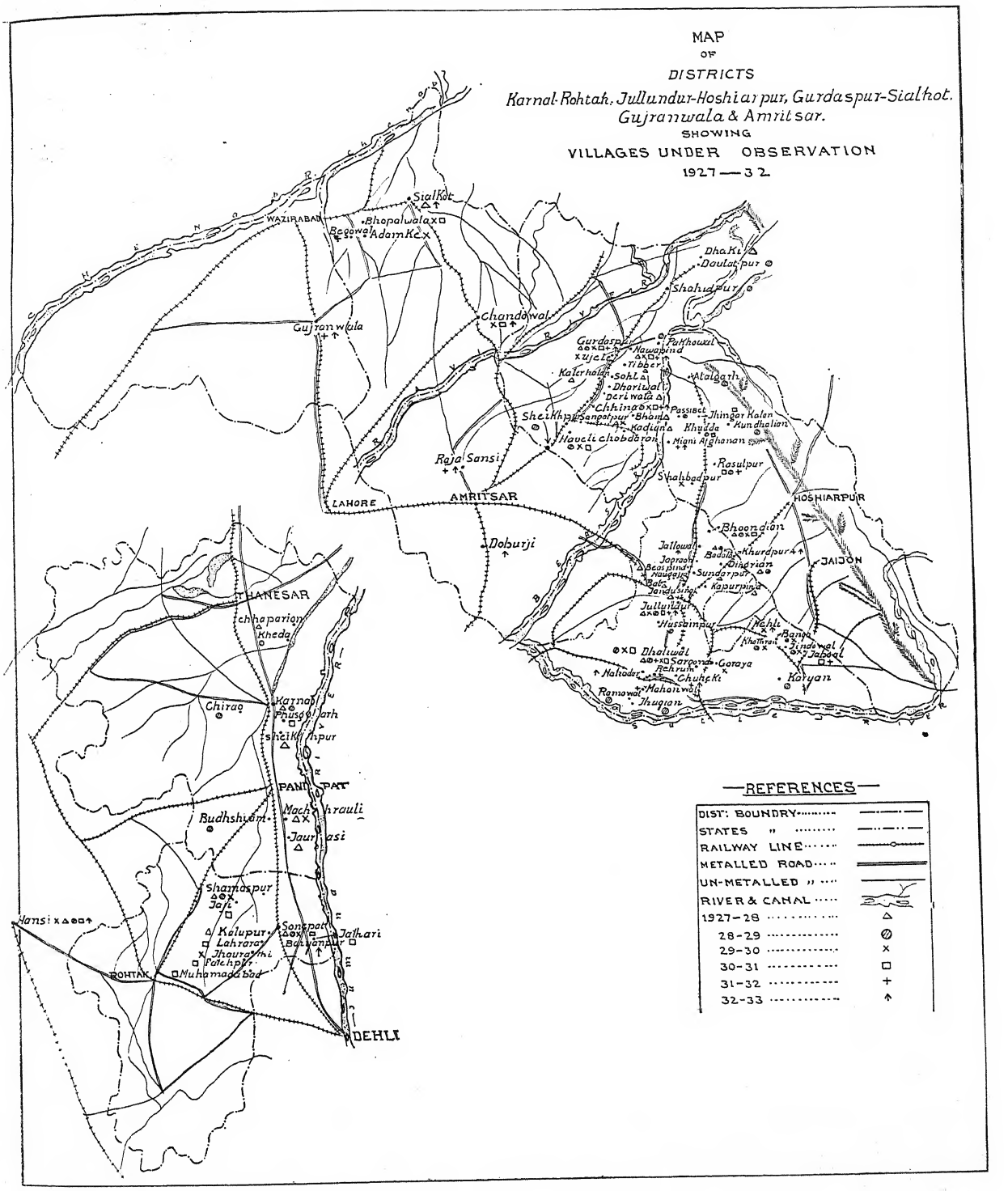
A chemical assistant was posted to each and arrangements made for representative types of selected canes to be analysed at fortnightly intervals throughout the cane season from November to the middle of April, both from the Government Farms and from the land of zamindars at various spots throughout the districts.

1. The South-Eastern Tract (Karnal and Rohtak districts)

(*a*) *General physical and climatic conditions.*—Karnal and Rohtak which contain the largest areas under sugarcane of the south-eastern cane growing districts of the Punjab belong to the Western Gangetic Plain, with a mean annual rainfall of about 24 inches. The soils of this area consist mostly of medium loams, which are sufficiently retentive of moisture, and, given adequate irrigation, are extremely fertile.

The consumption of sugar in this area is high and there is a big local demand both for raw and refined sugar.

Frost is not as common an occurrence as in some other parts of the Province, and thus this tract offers very suitable conditions for cane growing. The high temperatures, combined with the high humidity, which are experienced are very favourable for the growth of cane during the monsoon period, but unfortunately, these conditions are limited in duration, and are offset to some extent by the earlier hot dry months, and the succeeding cold season.


MAP
OF
DISTRICTS
Karnal-Rohtak. Jullundur-Hoshiarpur, Gurdaspur-Sialkot.
Gujranwala & Amritsar.
SHOWING
VILLAGES UNDER OBSERVATION
1927—32.
SialKot
WAZIRABAD
Bhopalwala
Begowal
Adam Ke
Gujranwala
Chandowal
Dhaki
Doulat pur
Shahdpur
Gurdaspur
Pathkowal
Nawapind
Tibber
Kalerhalan
Sahl
Dhoriwala
Derkwala
Chhina
Sanpatpur
Bhoma
Kadian
Sheikhupur
Haveli chobdaran
Passiaci
Jhingar Kalan
Kundhalian
Khudda
Miani Afghanan
Malgath
Raja Sansi
Rasulpur
Shahbadpur
LAHORE
AMRITSAR
HOSHIARPUR
Doburji
Bhoondian
Jallowal
Budala
Khurdpur
Jagraon
Dhadrian
Sundarpur
Kapurthala
JAIJON
Jullundur
Hassanpur
Mehli
Banga
Khatkarkalan
Jindowal
Jaboal
Dhaliwal
Sarpond
Goraya
Nahodar
Chuhekhi
Karyan
Ramowal
Mahalwa
Jhugian
HANESAR
Chhaparian
Kheda
Chirao
Karnal
Pugsoth
Shekhpur
PANIPAT
Machhrauli
Budhshiam
Jaurasi
Shamaspur
Jafi
Hansi
Kalupur
Lahrara
Sonepat
Bahlumpur
Jathari
Jhaurasi
Pabnawa
ROHTAK
Muhammadabad
DEHLI
REFERENCES
DIST: BOUNDRY
STATES "
RAILWAY LINE
METALLED ROAD
UN-METALLED "
RIVER & CANAL
1927-28
28-29
29-30
30-31
31-32
32-33

These climatic factors with extremes of temperature necessarily impose certain limitations on the yield and quality of cane produced.

(*b*) *Agricultural features.*—As the cane growing tracts of Rohtak and Karnal are only separated by the River Jumna from the corresponding districts of Meerut and Muzaffarnagar of the United Provinces, they show similar climatic factors and the general agricultural methods employed are essentially the same.

The soils are alluvial, varying from some stiff clays in the Karnal district to clay and medium loams in a more southerly direction. Hence with a well distributed rainfall of 25 inches per annum, supplemented by canal or well irrigation they are eminently suitable for cane.

The rotations usually followed are :—

1. Wheat—>Maize—>*Metha* and Sugarcane.
2. Sugarcane—>Wheat—>*Jowar*, *Bajra* or *Gowara*, and Gram.
3. Sugarcane—>Wheat—>Chillies or Cotton.
4. Sugarcane—>Wheat—>*Jowar*, *Bajra*, or Cotton and *Metha*.
5. Sugarcane—>Wheat—>Cotton, Wheat or Wheat+Gram (mixture).

Sugarcane and wheat are invariably manured, farmyard manure being used and applied to every crop when available. In a normal year cane receives from 400 to 600 mds. of manure per acre, from 10 to 15 irrigations, and is intercultured from 6 to 10 times with hand implements.

(*c*) *Varieties of cane grown.*—Most of the local varieties ripen early. Lalri, a thin cane, resembling Katha, more common to the Central districts, is extensively grown on well and canal irrigated land in the Karnal, Rohtak and Gurgaon districts. It has a good tillering capacity, is a hard frost resistant cane and is capable of surviving conditions which would be fatal to medium or thick varieties. The yield in juice is lower and the cane more fibrous than Suretha, but the juice has a high sucrose content.

The outturn is from 15 to 18 tons of cane per acre, giving about 1·5 tons of *gur*.

Suretha is a thicker, softer and whiter cane than Lalri, requires more irrigations and is liable to suffer from drought, but under suitable conditions it attains a considerable height and yields about 20 tons or even more per acre.

Dhaulu, which is a white, soft, chewing cane, is usually grown with Bodichin under *barani* conditions. Its average yield per acre is 25 tons of stripped cane yielding from 2 to 2·5 tons of *gur*, which fetches a high price in the market.

Manure is not usually applied to Dhaulu, which follows the rotation Cane—Fallow—Cane. This variety, however, is particularly susceptible to frost and pests, and suffered very severely in the 1928-29 season on this account.

Of the Coimbatore varieties, Co. 205, Co. 213, and Co. 223, were usually grown on an area which was increasing year by year.

Co. 205 gives a comparatively low sucrose content and high glucose ratio, possesses a long deep root system and will tolerate the disadvantages of *barani* tracts, poor and indifferent cultivators, and is immune to most of the prevalent diseases of cane.

Its yield in this locality has been about 2·5 tons of *gur* per acre, although far greater yields have been obtained at the Lyallpur Agricultural Station.

Co. 213 and Co. 223 resemble each other as regards their sucrose and glucose. content and high yields which amount to about 3 tons of *gur* per acre, provided sufficient manure and watering is given, but otherwise the yield is much lower, Co. 223 is, however, a soft cane and consequently liable to damage by jackals and other animals, and on this account it has lost favour with the cultivators to such a degree that during the 1930-31 season, it was not grown on any appreciable scale, while the area under other improved varieties steadily increased.

Other varieties, such as Co. 300, and Co. 312, have also been introduced in these localities. The Coimbatore varieties, in addition to their high yields possess considerable power of ratooning, and this is an additional reason for their increasing popularity.

(*d*) *Period of optimum ripeness.*—Harvesting generally begins by the middle of November, and is continued till the end of February, although, occasionally, when winter rains delay ripening, it extends well into March.

The above-mentioned varieties provide for a crushing season of about 4 months which is requisite for the successful working of a factory, although the average zamindar prefers to clear his fields at the earliest date possible. The period of optimum ripeness, the requirements of the zamindar and the needs of the factory require, therefore, careful adjustment if the best all-round advantage is to be obtained.

The introduction of high yielding new varieties is no doubt helping the zamindar to extend the period during which his cane can be left standing, but in order to conform to general agricultural practice in these localities, an extension of the crushing season must be combined with the introduction of early ripening varieties, which owing to high yields and frost resisting qualities will be able to replace the local Lalri.

Analyses of different varieties of cane during the years of the survey show that canes in a mature condition fit for crushing are available by the first week of December and may continue so until the first week of April (Table VIII).

Co. 300, Co. 301, and Co. 313 are early ripeners and have done very well in Jullundur and Gurdaspur, and there appears to be no reason why they should not do equally well or better in the more equitable climate of the south-eastern Punjab to which they have recently been introduced, although analytical data have not yet been obtained.

(*e*) *Quality of the cane.*—Quality is an important consideration in factory requirements. Not only is it necessary to have a cane with a low fibre content, but as thick a juice as possible is desirable from the point of view of fuel consumption. A high purity coefficient and a low glucose ratio are also necessary, otherwise the sucrose fails to crystallize out properly as white sugar.

These factors are revealed in Tables I—VII of the analytical data collected during the years of the survey. The canes were analysed at fortnightly intervals from November to the middle of March and the figures shown are the averages of those obtained during the periods of optimum ripeness.

(*f*) *Comparison of indigenous and Coimbatore varieties.*—From the data collected it is easy to interpret the value of the two types of cane, which is summarised in terms of comparative average figures below :—

Outturn in tons per acre

	Stripped cane	Juice	Total solids	Sucrose	Glucose ratio	Purity coefficient
Local . .	24·60	12·70	2·42	2·01	1·50	84·38
Coimbatore .	26·67	15·13	2·93	2·42	2·16	86·05

It is clear that the Coimbatore varieties are in every way superior to the local varieties.

The glucose ratio is certainly higher, due to the inclusion of Co. 205, which has an exceptionally high glucose ratio and, but for its high yielding capacity and ability to withstand unfavourable climatic variations, would never have become popular either for *gur* or sugar production. It is, however, being gradually replaced by better types.

(*g*) *Effect of frost.*—As already stated these districts do not usually experience severe frost, and it is an uncommon experience to find cane damaged on that account to any appreciable extent, although when the whole Province experienced a fairly long spell of severe frost in the 1928-29 season, its effects were felt on the cane crop in these districts, and these will be discussed later in connection with the canes from the central and sub-montane areas.

2. The Central Tract (Jullundur and Hoshiarpur districts)

(*a*) *General physical and climatic conditions.*—These two districts lie between 30° 57′ N. and 32° 58′ N., being bounded on either side by the Sutlej and Beas, and form what is commonly known as the Doaba.

This area contains about 17 per cent of the total area under cane in the Punjab and has for long been regarded as the focus of the sugar industry whose decline in past years was largely due to foreign competition in Indian markets.

In the Hoshiarpur district the northern half of which is a mountainous tract containing the Siwaliks, a series of low-lying rocks forming the Sub-Himalayas and consisting of tertiary sandstone, clay and boulder conglomerates, runs along its entire length, the long strip of land from 3 to 8 miles in width bordering on Jullundur known as the Sirwal, is the most fertile part of the district in which most of the cane is grown.

The land lying between the Sirwal and the river Beas, known as the Bet, is unirrigated, and in it cane is produced under '*barani*' or '*sailab*' conditions, rendered possible by an average rainfall of 30-35 in. and a soil which does not dry rapidly.

The Jullundur district, however, further away from the foothills of the Himalayas, is better and has an annual rainfall somewhat lower, *viz.*, 26 inches, and consists of a vast expanse of rich alluvial soil considered to be one of the best in the Punjab. The Dhak and Manjki tracts, which lie towards the south of this district, and the Sirwal tract just to their north, are the most suitable for the production of a sugarcane crop about 85 per cent of which crop is irrigated.

(*b*) *Agricultural practice.*—The Jullundur district is dependent very largely on well irrigation by means of Persian wheels, and the crop rotations generally followed are :—

1. Wheat—>Maize—>Cotton—>*Senji*—>Sugarcane.
2. Wheat—>Maize—>*Senji*—>Sugarcane.
3. Wheat—>*Chari, Guara*—>Sugarcane.

Farmyard manure is generally applied to maize and cane in the first rotation and to maize or *chari-guara* in the 2nd and 3rd rotations respectively, the usual rate of application being from 150 to 300 maunds per acre. In some localities ammonium sulphate applied to the various fodder crops, or to the cane itself, has been used with marked success, and Co. varieties invariably give the best returns when conditions of cultivation, irrigation and manuring are favourable.

Zamindars have now learnt to appreciate the necessity of an adequate supply of manure, and since the quantity of farmyard manure available is limited, castor cake has been tried in some places with success.

In the Hoshiarpur district most of the cane is unirrigated, being grown under '*barani*' or '*sailab*' conditions, although there is an innundation canal known as the

Shah-Nahr which irrigates parts of the Bet lands. No definite or fixed rotations are followed in this tract. In the Bet area ratooning is very commonly practised and it has been found that the second and third crops give better yields than the first. This practice is sometimes extended to the 6th or 7th year although the yield generally begins to fall off after the fourth year.

(c) *Varieties commonly grown.*—During the first year of this survey, 1927-28, the most common indigenous varieties grown were Ponda, Katha, Dhaulu, and Yuba, together with Kinara and Ekar to some extent, Katha everywhere predominated, however, and was the only cane grown under '*barani*' condition.

Ponda was grown chiefly near towns, but had never been used for sugar making ; Dhaulu was confined to '*chahi*' lands, while Yuba, owing to its great susceptibility to frost had almost disappeared.

The most important new varieties introduced have been Co. 205 and Co. 223, with Co. 213, as the latest arrival.

During the second and third year of the survey, 1928-30, although Katha was the only local variety found in any quantity in these two districts, some cultivators adopted the new Co. varieties to the entire exclusion of local ones, and inspite of some prejudice against it Co. 205 retained its position as it adapted itself better to ratooning and was not liable to attack of white ants, jackals, etc.

Co. 223, owing to its high yields, and producing a better quality *gur*, was very enthusiastically taken up to begin with. It showed, however, the disadvantages of having a characteristic hollow in the pith, and a split in the skin which rendered it liable to attack by pests and was gradually discarded, so much so, that by the time the survey was concluded it had almost gone out of cultivation except on poor lands. On the other hand Co. 213, owing to its good yield, high sucrose content, great vigour and excellent behaviour at maturity was enjoying an increased popularity.

In the 1931-32 season three more varieties Co. 281, Co. 285, and Co. 290 were investigated in more favourable parts of the district. Co. 285, in addition to being as high yielding as Co. 205, showed equal vigour and was also an early ripener with a low glucose ratio, and has in consequence largely replaced both Katha and Co. 205.

(d) *Period of optimum ripeness.*—Ratoon and '*barani*' crops which ripen early are generally the first to be harvested, thus Katha is ready by the middle of November and sometimes earlier and crushing is finished before the advent of frost.

The Coimbatore varieties, on the other hand for the most part, ripen late.

In tract 'B' including the Bet area of the Hoshiarpur district (Tract 'A' includes the Dhak and Manjki areas and tract 'B' includes the Sirwal and Bet lands around Tanda and the Government Agricultural Farm at Jullundur) frost is usually very severe, and unless frost resisting varieties are introduced for the later part of the season, a prolonged crushing season is not possible for factory requirements.

Part of the Jullundur Tehsil, and the whole of the area included in tract 'A' are well adapted for a long crushing season, with Katha and a correct selection of Co varieties.

The periods of optimum ripeness of the varieties of cane analysed during the entire period of the survey are shown in Table XVII.

It appears from this that in normal years crushing could start before the end of November and be continued to the middle of March, the actual period in any year being liable to vary according to the climatic conditions prevailing.

A prolonged dry season at the beginning of winter would tend to ripen the cane earlier and conversely a long spell of wet weather would retard ripening.

Thus in 1932-33 the season was absolutely dry from the first week of September to the third week of December, and a dry cold January and February succeeded with 28 frosty nights, the result of which was a crushing season which extended from November to the end of April, *viz.*, 175 days compared with 130 days in the previous year.

Manuring also has a decided effect on the ripening period, generally delaying it, and it is a common observation that most practices which in any way help to increase yields such as excessive water supply, manuring, etc., tend to delay ripening.

Thus in irrigated areas these two factors give the farmer a certain degree of latitude in controlling the ripening period of his crops.

(*e*) *Quality of the canes.*—The data presented in Tables IX to XVI show the yields per acre and the composition of the juice from the different varieties of cane analysed. As in the case of the Gurdaspur canes, it will be seen that the composition of any variety varies only within very narrow limits from year to year, unless of course, a cane be badly attacked by disease or pests, as was the case with Co. 213, Co. 281, and Co. 285 at the Jullundur Agricultural Farm during the 1930-31 season and with Co. 223 at Khothran when the sucrose percentage fell as low as 5·7 (*see* page 233).

Very considerable differences in tonnage yield are, however, experienced with the same cane from year to year, or at different localities in the same year. For example the average yield of Co. 223 was 15·4 tons in 1928-29 as against 26·83 in 1931-32, the reason apparently being that the former season was a uniformly bad one for the cane crop while the 1931-32 season was a very good one.

Again in 1928-29 the yield from this variety varied from 8·6 tons at Jindowal to 23 tons at Nawapind, while Katha varied from a yield of 8·9 tons at Sargondi to 20·3 at Bhoondian, due to what may be termed 'local causes'.

Taking into consideration these differences in yield the average figures given in Table XV show representative 'normal' yields, such as may be expected for the locality, unaffected by adverse or very favourable conditions.

(*e*) *Local and Coimbatore varieties.*—Table XVI shows the comparative figures for the different local and imported varieties averaged over a number of years, and as the following figures indicate the new varieties are very superior to the local ones.

Outturn in tons per acre

	Stripped cane	Juice	Total solids	Sucrose	Glucose ratio	Purity coefficient
Local (Katha) .	15·80	9·01	1·71	1·43	2·1	83·1
Coimbatore .	24·09	15·68	2·89	2·45	3·3	84·6

As was found in the southern Punjab, the glucose ratio is higher for the Coimbatore than for the local varieties, and for the same reasons, *viz.*, owing to the inclusion of the inferior Co. 205, and here also, this cane is disappearing as better varieties become available.

3. The Sub-montane Tract (Gurdaspur and Sialkot districts)

This circle comprises the districts of Gurdaspur, Sialkot, Gujranwala and Amritsar and is one of the largest cane tracts in the Punjab, growing about one third of the total cane of the Province or about 5 per cent of that grown in the whole of ndia.

The soil of this circle is a medium loam of average fertility, although in some parts of the Gurdaspur and Sialkot districts, which constitute the sub-montane tracts, the soil is very badly drained, and alkali conditions are commonly met with in the Gujranwala and Amritsar districts. The rainfall which occurs mostly from July to September averages from 30 to 40 in. per annum, and increases towards the hills, while frost is of annual occurrence and damages the crop according to its intensity.

The riverian tracts of the Beas and Ravi rivers are especially noted for *sailab* canes, and only a few of the newer selected varieties such as Co. 205 and Co. 285, which can withstand water-logged conditions, can be grown in such localities ; as a result of the environment however, these have become very soft and the yield varies greatly being very low, for example in the Pathankot Tehsil as compared with Batala further south.

(*a*) *Agricultural practice.*—As already noted Gurdaspur is one of the largest cane growing districts of the Province, most of the cane being produced under '*barani*' conditions although well and canal irrigation is also practised.

The '*barani*' area includes the *sailab* lands which are a very interesting feature of the district as regards the sugarcane crop.

The most common rotation followed on these lands is sugarcane—sugarcane, or sugarcane—wheat, farmyard manure being applied only to the cane.

In the Tehsils of Batala and Gurdaspur the usual rotation followed, both on canal and well irrigated lands is, wheat—maize—*senji*—cane, the manure being applied to the maize only as a rule, at the rate of about 200 maunds per acre, although when available a little is also applied before sowing cane.

The cultivators of this district, apart from those of the Batala Tehsil are, generally speaking very indifferent and take little pains to cultivate the fields properly, with the result that low yields of cane are a common feature.

Under such inadequate and superficial conditions of cultivation the cane plant develops a shallow and inferior root system, a condition intensified by an excess of moisture, with the result that the cane frequently lodges. The percentage of fibre thus increases and that of juice falls, and both quality and yield are therefore adversely affected.

(*b*) *Varieties of cane.*—The history of different cane varieties met with in the Gurdaspur district presents some interesting features.

During the first year of the survey in 1927-28, Yuba, Dhaulu, Katha, Co. 205, and Co. 223, were the chief varieties grown, with Kansar, Kahu, Reora, and Kenara to a lesser extent.

Yuba is a soft cane and was grown chiefly owing to its superior quality and high juice content. Owing to its susceptibility to attack from insects and diseases, however, it has gradually been displaced by Co. 205 and Co. 223.

Dhaulu was generally grown on a rich loam soil, having a good water supply, while Katha was, and still is, grown extensively under *barani* conditions. Katha owes its survival to the superior quality *gur* produced from it, and to the fact that it will withstand the effects of frost better than many other local varieties. Otherwise, owing to its low tonnage yield, and high fibre content it would have disappeared long ago.

Co. 205 owes its popularity to its being a thick skinned hardy cane, able to withstand frost, drought and water-logging, but it has the disadvantage of giving a low quality juice with a somewhat brackish taste.

Co. 223 is free from this defect and gives good yields, but it is soft and juicy and is thus liable to attack by insects and wild animals.

By the year 1929-30 Co. 285 had also become established at a number of places.

Succeeding years have seen a steady increase in the area under Coimbatore varieties, the extension apparently being limited by the supply of seed available, so that in 1931-32, Co. 213, and Co. 285 were grown extensively while Co. 205 and Co. 223 were losing favour. During succeeding years Co. 285 continued to be widely grown together with Co. 213, Co. 290 and Co. 300 while Co. 205 and Co. 223 had almost entirely disappeared. This obviously indicates that the zamindar is willing

rapidly to adopt improved varieties once he is convinced of their superiority and the higher profit accruing to himself by so doing.

In the Sialkot district, where, as late as 1929-30 the chief varieties grown were the local canes, Dhaulu, Katha, Kahu, or Maitku, the Coimbatore varieties have made rapid headway; in 1932-33 the last year of the survey, Co. 205, Co. 223, Co. 285, and Co. 290, were largely grown, and there is every indication that the zamindar is only too willing to replace low yielding indigenous varieties by better types, to the extent that seed of the latter is available.

(c) *Period of optimum ripeness.*—The ripening period generally extends from the middle of December to the end of March, although local zamindars start crushing for *gur* manufacture a month earlier.

It will be seen from Table XXVI that Dhaulu is an early ripening variety, and so may be judiciously employed to assist earlier crushing. None of the Co. varieties ripens as early as Dhaulu. Co. 285 and Co. 290 are not ready till December, so the introduction of such types is obviously necessary if the crushing season is to be extended. The types chosen must, however, be frost resisting as far as possible.

If these canes are kept standing beyond the end of March, speedy deterioration sets in, except when the winter is unduly prolonged, as was the case in 1930-31, when ripening was considerably delayed in consequence.

It is possible now by a correct selection of types to assure a crushing season of 120 days extending from the first week of December to the last week of March (Table XVII).

Quality of canes.—The analytical data on the different varieties of cane investigated during the survey are shown in Tables XVIII to XXIV, the figures given being as in the case of the south eastern Punjab, the averages of those found during the period of optimum ripeness. It will be observed that, with the exception of those canes which suffered from disease, such as red rot, or had been affected by frost at the time of their maximum ripeness, the composition of the juice varied only within narrow limits, and may be said to have remained practically constant in different seasons. The chief factors responsible for varying tonnage yields are the soil, cultivation, manuring and the effect of the seasons.

Thus the yield of Co. 205 varied from a minimum of 11·9 to a maximum of 22·7 tons, that of Co. 223 from 15·6 to 20·2 tons, that of Co. 285 from 16·4 to 22·25 tons and of Co. 290 from 16·7 to 21·3 tons per acre.

Tables XXV and XXVI show at a glance information useful alike to the grower and the manufacturer.

(*d*) *Indigenous and Coimbatore varieties.*—In the following table are shown the average figures for all the local and Coimbatore varieties analysed at all the different localities during the period of the survey :—

Outturn in tons per acre

	Stripped cane	Juice	Total solids	Sucrose	Glucose ratio	Purity coefficient
Local . .	12·02	7·15	1·29	1·09	5·16	83·1
Coimbatore .	17·90	11·16	2·06	1·77	4·87	86·0

The advantages of the Co. varieties are more pronounced in this tract than in the case of the Karnal and Rohtak districts, the outturn per acre in stripped cane, juice, total solids and sucrose of the new varieties being more than 50 per cent in excess of the figures from the local varieties, as compared with an excess of only about 20 per cent in the South Eastern Punjab.

This smaller difference of 20 per cent. in favour of Coimbatore varieties in the South-Eastern Punjab is largely due to the influence of Suretha which is not only one of the best local varieties of the province but compares favourably with the improved new varieties. The low average glucose ratio of the new varieties in the Gurdaspur area is due to the inclusion of Co. 214 which has a very low glucose ratio *viz.*, 1·9, and which, but for its low yield would undoubtedly be one of the best early ripening canes.

The effect of frost on sugarcane

From a climatic point of view, the Punjab does not appear to be pre-eminently suitable for the cultivation of sugarcane, as it lies between 27° 39″ and 34° 2″ N. and experiences a range of temperature from below freezing point in December and January to a maximum shade temperature of 118°F. or more at Lyallpur in May and June.

During the ripening period cane shows a progressive increase in the sucrose content with a corresponding decrease in invert sugar or glucose which continues till the cane is mature.

The effect of frost at any time during this period not only checks ripening but reverses the above process, although not all varieties are equally affected, and some recovery may take place if the frost is not too prolonged or severe.

Sugarcane may thus be divided into three groups depending on their reaction to frost :—

(*a*) Frost-resistant (unaffected or almost unaffected by cold),

(*b*) Semi-resistant [these include varieties which are either (1) slowly affected by frost or (2) quickly affected but also quickly recovering from its ill effects],

(*c*) Non-resistant (varieties which are quickly affected and which either fail to recover or recover very slowly).

It is most important therefore in the Punjab to select as far as possible frost-resistant varieties or the benefits of an otherwise good season may be largely nullified at a critical period if frost supervenes.

Special attention has accordingly been directed during these surveys to the collection of data which will enable different varieties of cane to be classified according to their capacity to resist frost and the following have been specially investigated :—

Desi.—Uba, Katha, Dhaulu, Lalri and Suretha.

Coimbatore.—Co. 205, Co. 213, Co. 223, Co. 270, Co. 281 Co. 285, and Co. 290.

Some account will now be given of the effect of frost on these canes.

During the first year's survey (1927-28) there was no frost in the Karnal and Rohtak districts, nor any of sufficient severity or duration in other districts surveyed to affect the cane.

In the succeeding sugarcane season, *i.e.*, 1928-29, very severe weather, with continued frost occurred at a time coinciding with the maturity period of the cane, and adversely affected its maturing throughout the Province.

In the year 1928-29 the minimum temperatures (in shade and at a height of 4 to 5 ft. above the ground level) recorded at Gurdaspur, Jullundur, and Karnal were 27·7°C., 30°C., and 38°C., respectively. (It must be remembered that the actual minimum temperature experienced by cane is always considerably lower than the minimum as recorded by the thermometer.) The dates on which frost actually occurred are shown below.

	December	January	February
Gurdaspur . . .	27, 29	7, 21-22, 31	1-2, 4, 6, 13, 15
Jullundur	27—30	6—10, 21—23, 31	1-2, 12
Karnal	6, 19-26, 31	1—3, 5—11, 31	1-2

The effect of sustained frost adversely affected both the physical condition of the canes and the quality of the juice. The leaves withered, buds were killed and the cane was rendered quite unfit for purposes of seed.

The effect on the quality of the juice was different according to the variety. Early ripening varieties suffered inversion of the sucrose, *i.e.*, conversion into glucose and a return to what might be considered a normal sucrose content was rarely attained.

This may be seen from the analyses of Dhaulu and Katha shown in Tables XXVII and XXVIII for the year 1928-29.

During mild frost in December and January, the sucrose-glucose ratio in the Coimbatore canes in general showed irregular fluctuations, indicating the possibility of recovery. Succeeding sharp attacks on January 31st, February 1st and 2nd, 1929, rendered future recovery out of the question however, except in the case of certain varieties which showed some improvement.

Of the three varieties, Co. 205, Co. 213, and Co. 223, which alone, amongst the Coimbatore canes, were available in a sufficiently large number of localities, Co. 213 in most cases showed almost complete immunity, being followed by Co. 205 and Co. 223 in this respect. If, however, we group together the number of cases in which these latter two canes showed either resistant or semi-resistant qualities there is little to choose between them.

In the South-Eastern Punjab mild attacks of frost appeared not to have affected the canes, but the severe attacks on 31st January and 1st and 2nd February 1929 did adversely affect them.

The early ripening variety, Dhaulu, and also Larli and Suretha at some places, however, were so badly affected that they were unable to recover, while Co. 205, a late ripener, in three cases out of four, and Co. 213 and Co. 223 in 50 per cent of the cases investigated recovered completely.

At the Gurdaspur Agricultural Station it was noticed that Co. 281, an early, ripener, is one of the best frost resistant canes, while Co. 285 and Co. 290, which ripen in January, were also able to recover from the ill effects of frost.

In the 1929-30 season cane in the Gurdaspur district suffered more from hail in October than from mild frost experienced later on, while no appreciable permanent ill effects were noticed in the Sialkot and southern districts.

In the central districts, *viz.*, Jullundur and Hoshiarpur, the weather was warmer during the ripening period than in the same period of the previous year, although there were 24 frosty nights. Severe frost from the 19th to the 21st January, however, caused a reduction in the sucrose content, but the canes on the whole recovered.

In 1930-31 frost occurred intermittently from early December till the end of February and its effects were intensified by the previous dry weather. The lowest temperatures recorded were 28°F. at Sialkot on 1st January 1931, 29·6°F. at Gurdaspur on 22nd December 1930, and 28°F. at Jullundur on 22nd and 26th December.

It has been observed that the degree of frost a cane can withstand is modified to some extent by the nature and moisture content of the soil, and that canes growing on light loams suffer less than those on stiffer soils. Furthermore an application of potassium nitrate as a manure conferred some degree of immunity.

In the Jullundur and Hoshiarpur districts the monthly average maximum temperature from May to April was higher, and the average minimum lower than in the two previous years. The Sirwal and Bet lands near Tanda experienced the heaviest frost of the season and Katha cane at Jabboal suffered heavily in early December, when its glucose content increased from 0·11 per cent on December 8th to 0·54 per cent on the 19th December. This and succeeding attacks so damaged the cane that the glucose content never recovered to its original low value (Table XXIX).

The Coimbatore varieties, however, particularly Co. 213, which ripened late, after the frost, showed but little ill effect, although Co. 205 in five cases out of fifteen, and Co. 223 in one case out of four succumbed. It may be pointed out, however, that in the case of Co. 205, the crop (ratoon and plant) at Jhingar Kalan (fig. 9) and Bhondian as in the case of Co. 223 at Rasulpur experienced the sharpest frost in the district, while Co. 205 at Lahrara was infested by Pyrilla (fig. 9).

The winter of 1931-32 was comparatively mild throughout the Province, the lowest temperatures in the area surveyed being 30°F., 33°F. and 30·5°F. at Gurdaspur, Sialkot and Jullundur, respectively, and canes suffered very little damage beyond a temporary set back in some cases in February.

The winter of 1932-33 was unusually long with severe frosts at Gurdaspur and Sialkot from early December till the end of January, but these were tempered to some degree by occasional rain.

A noticeable effect of the prolonged cold was that most of the canes affected by it recovered in February or March and it appears that the injury caused by frost is aggravated if it continues very late in the season, when it is usually rapidly succeeded by abnormally warm weather.

When, however, moderately cold weather succeeds hard frost, the affected canes appear to have a better chance of recovery.

These conclusions are confirmed by observations made in the Jullundur and Gurdaspur districts in 1930-31, where, at many of the places surveyed Co. 223 ripened as late as the end of March, while at the Agricultural Station, Gurdaspur, Co. 223 (both the manured and unmanured crops) ripened as late as April (Tables XXX and XXXI and Fig. 12).

Similarly in 1932-33 at Chandowal, Katha, an early ripening variety, attained its maximum purity in the first week of April (Table XXXII and Fig. 2).

In the Jullundur and Hoshiarpur areas a timely fall of 2 in. of rain in the last week of December and early January succeeding a prolonged drought somewhat mitigated the effects of 28 nights of frost in January and February.

The bad effects, however, were most noticeable on Katha and Co. 300, both early ripening canes and caused a set back of a month in the crushing season.

Other varieties also received a temporary set back, particularly Co. 213 at Nakodar, Co. 205 at Miani Afghanan and Khurdpur, and Co. 285, Co. 301 and Co. 313 at Jullundur, while Co. 281 again showed the best resistance.

From these observations made over a period of six years it appears that the capacity of canes to escape permenent damage by frost depends in the first place on the intensity and duration of the attack, and is also related to the climatic conditions both before and after the same. Furthermore there is a greater likelihood of canes recovering from severe frost early in the season than there is from frost later on when the canes are ripe or nearly so. Generally speaking it may be observed that no variety is perfectly immune to the ill effects of frost under all conditions. A cane's behaviour as a resistant, semi-resistant or non-resistant type during any season will be determined by the local conditions prevalent at the time. Thus we find every variety, in canes where the number of observations have been sufficient, exhibiting the behaviour of all the above three types. A few selected canes are illustrated in Figs. 1—16.

The condition of the cane before frost occurs is an important factor in relation to the effect produced. Healthy canes unaffected by disease or previous drought will be more likely to recover than those which have been adversely affected in these respects. Thus a cane subjected to a combined attack of frost and Pyrilla will seldom recover, as was seen in 1930-31, when almost all varieties examined in the village of Lehrara in the south Punjab were attacked by Pyrilla, and mild frosts which supervened in December or subsequently proved fatal (Tables XXXIII—XXXV, Figs. 3, 5 and 9). Similarly in 1932-33, Co. 213, Co. 223, and Co. 300 suffered badly from red rot at Raja Sansi with the result that when frost followed they were never able to recover and the juice was of a disagreeable odour (Tables XXXVI—XXXVIII).

The effect of irrigation and rain in moderating the effect of frost is well known.

If the cane crop receives water immediately preceding or at the beginning of the onset of frost it either does not suffer at all or if it does it can recover in course of time with little worse effect than a delay in the time of maturity.

For example in 1929-30 in the Jullundur district apart from the mild frost at the end of November each subsequent frost was preceded by rain with the result that the crops did not suffer to any appreciable extent, while on the other hand canes attacked by Pyrilla appeared to improve, as a result of the insect having been dislodged from the leaves by rain and subsequently killed by the intense cold. Conversely the bad effect of frost in the absence of water is illustrated in the case of Katha canes at Khudda (*sailab* lands of Hoshiarpur) which did not suffer to so great an extent from the severe attack of frost in 1928-29, as it did from a comparatively

mild attack in 1930-31 (Tables XXVIII and XXIX). The explanation of this differential behaviour seems to lie in the fact that the locality received more than 5·35 in. of rain in 1928-29 distributed as shown below while in 1930-31, there was a dry period lasting practically from October to December, and a fall of 1·03 in. of rain in January did not prove sufficient to mitigate the ill effects of frost in the previous dry months.

——	Oct.	Nov.	Dec.	Jan.	Total
1928-29	0·21	2·52	1·78	0·84	5·35
1930-31	*Nil*	*Nil*	0·15	1·03	1·18

An illustration of the beneficial effect of rain not only in mitigating the ill effect of frost but also in prolonging the crushing period of cane may be seen on referring to Table XI. Katha is an early ripening, semi-resistant cane, inclined to succumb to a severe attack of frost. The frost in 1930-31 was one of the severest during the period of the survey and yet the cane withstood it successfully at Jhabian-wala near Jullundur city, because, during that year, rain and frost invariably alternated with each other. As will be seen 14 frosty nights from the 8th to 12th and from the 19th to the 27th of December affected the cane adversely, both by lowering its purity coefficient and increasing the glucose ratio. The injury to cane due to further attacks of frost on the 1st and 2nd January would very likely have been more severe from what we know of similar cases, but for the mitigating effect of 0·62 in. of rain on the 29th January. A fall of 1·62 inches of rain from the 21st to the 25th of January successfully counteracted the ill effects of eight frosty nights, on six of which the lowest temperatures of the season were recorded. This is evident from the fact that inversion of sucrose was negligible. Comparatively milder attacks of frost on the 29th and 30th of January preceded as they were by 1·62 inches of rain from the 21st to the 25th of January were unattended by any ill effects. Further rain in February, although it did not produce immediately favourable effects, was followed by rain in March, and improved the cane so much that on the 11th of March it attained the highest purity and lowest glucose ratio of the season.

Other similar harmful effects of frost without irrigation may be seen in the case of the canes at Jhingar Kalan (Tables XLI to XLIII), while the beneficial effects of irrigation are well shown in Tables XLIV relating to the Government Agricultural Farm at Jullundur.

The village of Rasulpur near Tanda Urmur on the Jullundur-Mukerian line usually grows its cane under well irrigation, but in 1930-31 the Co. 223 crop reserved for analysis received no water during the period of frost owing to the well from which it was irrigated being out of action. This factor, combined with an absence of rain in October and November injured the cane beyond recovery (Table XLV).

On the other hand, in the season 1931-32, when long continued frost occurred the same variety of cane at the same place received normal irrigation and matured in a normal manner (Table XLVI).

During exceptionally cold weather, almost all varieties are affected to some extent, although the resistance to moderate cold varies. Tables XLVII and XLVIII show the distribution of different varieties of cane (*viz.*, frost-resistant, semi-resistant, and non-resistant) in all the localities surveyed. As will be seen, this presentation is the result of a consideration of the behaviour of different varieties of sugarcane in 384 individual cases. It also shows that of the *desi* varieties non-resistant types of cane constitute about 37 per cent of the whole and the worst sufferers from frost were Katha and Dhaulu, followed by Suretha and Lalri. Of the indigenous varieties Lalri is one of the best frost-resistant types. Of the Coimbatore varieties, Co. 270 and Co. 281 may be considered resistant. Semi-resistant types, which will resist mild frost completely in most cases and only succumb under exceptionally severe attacks are Co. 213, Co. 285 and Co. 290, while Co. 205, and Co. 223 are definitely semi-resistant, Co. 205 * being slightly less so than Co. 223.

Thus on the basis of data available, the Coimbatore varieties may be arranged as follows, in order of decreasing resistance to frost:

Co. 270, Co. 281, Co. 285, Co. 290, Co. 213, Co. 223 and Co. 205.

Other varieties, *viz.*, Co. 300, Co. 301, Co. 312 and Co. 313 appear to be good frost, resistant canes, but the number of observations made on them is not yet sufficient to enable a definite expression of opinion to be given.

Diseases and pests

During the period covered by this survey the effects of a number of diseases and pests were studied, the chief among the later being, Pyrilla, Aleurodidae, top borer, stem borer, pyrallid borer, and white ants. Mosaic disease and red rot were also met with, but not in a virulent form.

Smut was particularly noticed attacking Katha canes at Nakodar in Jullundur, while aleurodidae and pyrallid borer were chiefly confined to the Gurdaspur circle, other diseases being more or less common to all the localities under survey.

Locusts

During August and September 1929 locusts did considerable damage to the cane crop of the Southern Punjab and central districts. The destruction of the green leaves causes great disturbance to the metabolic processes (elaboration of juice and carbohydrates) in the growing plant with the result that very poor yields were obtained in the affected areas.

* It must be remembered, however, that Co. 205 is a cane primarily meant for '*barani*' or '*sailab*' lands and on that account it will suffer more from the ill effects of frost than irrigated canes. This is a very likely reason for its position after Co. 223 in the above list.

Shoot and top borers

These pests were fairly active in all the districts surveyed, but especially destructive in the Southern Punjab during the 1930-31 season when the ratoon crops showed a greater susceptibility.

Pyrallid borer

This pest caused great damage to the cane crop of Gurdaspur in the 1931-32 and 1932-33 seasons, and the canes in the Bet areas suffered particularly in the former season.

Owing to the pith of the cane being attacked and eaten the stem becomes weak, growth is stopped, and the burrows made by the borers frequently become infested by red rot.

An analytical comparison of healthy canes with those afflicted by this pest as given in Table XLIX, is most instructive.

As is seen from this table the sucrose content was diminished by about 33 per cent in the diseased canes, while glucose increase from 300 per cent to 400 per cent. It is difficult to clarify the juice obtained from diseased canes, which remains a deep yellow colour inspite of all ordinary clarifying agents, while the *gur* obtained from them is of an inferior quality.

Pyrilla

This pest was commonly found throughout the Punjab, and was responsible for serious damage wherever it appeared, although the central districts suffered most. In the 1930-31 season it was prevalent to such a degree that it was difficult to find any cane unattacked, and some idea of the extent of the depreciation of cane caused thereby may be seen from Table L which gives comparative data for the average yields which were obtained in the Southern Punjab for the year in question and the preceding year, when the crop was practically free from Pyrilla.

These figures are self-expanatory and illustrate the enormous damage which this pest can cause.

Pyrilla was generally found to be the most prevalent pest in the Jullundur and Hoshiarpur districts, one of the most afflicted villages seen being Shahbazpur in the Bet lands.

One plot of Co. 223 at Kothran which was badly attacked in the season 1929-30 gave the following figures on analysis.

Percentage on cane				Glucose ratio	Purity coefficient
Juice	Total solids	Sucrose	Glucose		
64.8	7·7	5·70	·81	15	74

The year 1930-31 was favourable for ascertaining the effects of this pest, as there were four varieties of cane, Co. 213, Co. 223, Co. 281 and Co. 285 all badly affected by Pyrilla on fields 2 and 3 of the Government Agricultural Farm at Jullundur, while in fields 13 and 14 in another block not far away the same varieties of cane (except Co. 223) were comparatively free. It was found that although the Pyrilla-affected canes gave a greater percentage of juice and glucose, the healthy crop gave more sucrose and total solids (Table LI).

The unaffected crop here gave 27 per cent more sucrose than the affected one, but the latter showed a glucose ratio three times greater than the former.

Co. 223 appeared however to be more susceptible to attack in both these areas.

This strange phenomenon of immunity and attack in almost adjacent areas appeared to be due to the fact that the crop in fields 2 and 3 under direct cultivation was a much heavier and a better one than the crop in fields 13 and 14 under tenant cultivation—the respective yields being 30·2 and 21·6 tons per acre and so provided better conditions for the protection and multiplication of the insects. Experience has shown that rain can destroy this pest and prevent undue damage if it falls at the right time, but it is, of course, a factor which cannot be controlled.

Conspicuous features of the effects of Pyrilla are that the quality of the crop suffers more than the tonnage yield, and that certain varieties appear to be more susceptible to attack than others. For example Katha, Co. 205 and Co. 285 appear to be more immune than are Co. 213 and Co. 223.

Aleurodidae

Aleurodidae are insects, the nature of whose attacks and effects are similar to those of Pyrilla. It has been found to be prevalent chiefly in the Gurdaspur district in all years of the survey except 1931-32.

White ants, rats and jackals

The depredations of white ants are well known, and they appear to show some preference for Co. 281, Co. 223 and to a certain extent for Co. 290, although no variety is immune.

Rats are serious enemies of the cane crop, and find shelter in heavy crops which have lodged, although lodging can be mitigated to some extent by growing the cane in trenches, which helps to prevent lodging and thus indirectly checks the rat menace.

Jackals are chiefly found in the Bet lands throughout the Province.

Smut and mosaic disease

Smut, which is a fungoid disease, has been noticed particularly in the Jullundur district but does not appear to have done any serious damage.

Mosaic, a virus disease, has the reputation in tropical countries of being responsible for great damage. Evidence of the disease which is more common in ratoon

crops is afforded by the leaves, which present a mottled appearance due to the formation of translucent yellow spots.

This disease was specially studied at Gurdaspur for three years and, as the figures in Table LII indicate, did not appear to cause any material deterioration in the composition of the juice, nor was the tonnage yield appreciably affected.

Red rot (*Colletotrichum falcatum* Went)

A serious attack of this disease which occurred during the season of 1932-33, on the cane crop of Rajah Sansi in the Amritsar district was studied.

All the varieties cultivated, *viz.*, Co. 213, Co. 223, Co. 285, Co. 290, and Co. 300 suffered badly and as a consequence the canes never reached their maximum maturity and deteriorated seriously after the attack, with the result that no *gur* could be made and the whole crop had to be burnt. Table LIII of analytical data obtained before and after the attack shows the effect of this disease and it will be noted that Co. 223 was damaged most and Co. 285 was the least affected. It may be noted that Co. 285 has proved as a rule to be one of the best frost and disease resisting varieties so far introduced.

FLOWERING OF SUGARCANE

Sugarcane does not usually flower in the Punjab although under certain favourable but undefined conditions Coimbatore canes do, but the old varieties such as Dhaulu and Katha hardly ever do so.

The zamindar usually regards this phenomenon as inauspicious and believes that flowering signifies a deterioration in the quality of the cane. If, however, we examine the analytical figures for the juice from flowering and non-flowering canes of the same variety (Table LIV) it will be seen that contrary to this belief the flowering canes are superior; they contain more sucrose, less glucose, and consequently have a lower glucose ratio and a higher purity coefficient.

The average figures for these four factors for Co. 205 in flowers was found to be 16·6, 0·61, 3·7 and 85 respectively, and 15·5, 0·78, 5·0 and 83 for Co. 205 not in flowers. Corresponding data for Co. 285 are shown in the table below :—

——	Sucrose	Glucose	Glucose ratio	Purity coefficient
Flowering	17·0	0·36	2·1	88
Flowerless.	16·5	0·40	2·4	87

The mild weather conditions of 1931-32 were very favourable for the cane crop and for observing the effects of flowering. Both Co. 205 and Co. 285 flowered at a

number of places in the Jullundur, Hoshiarpur, Ludhiana, Gurdaspur, and Gujranwala districts and a reference to Table LIV shows that the average weight of cane in flower was greater than that of a similar non-flowering cane, the figures being 580 grams against 442 grams for Co. 205, 803 grams against 616 grams for Co. 285, the excess weight averaging 30 per cent. It will be further noticed that flowering (or arrowing) does not affect the quality of the juice obtained, as the percentages of juice for the two types mentioned in flowering and flowerless cane were 57·9 and 59·2 for Co. 205, and 61·6 and 62·2 for Co. 285.

Table LV shows the monthly rainfall figures for the years 1927-28 to 1931-32 for the districts of Jullundur and Hoshiarpur, from which it will be observed that the rainfall and temperature during the four months, September-December, appeared to have a marked influence on the maturity and incidence of flowering in canes. Heavy rainfall during these months delayed maturity, while scanty rain accelerated it and checked vegetative growth, thus conducing to conditions favourable for flowering.

Abnormally high rainfall in the 1928-29 and 1929-30 seasons prevented the cane crop from maturing until as late as March. In the 1927-28 and 1931-32 seasons rainfall was deficient and the flowering of canes profuse.

From these observations it can be stated with reasonable confidence that not only are the fears of zamindars on the appearance of flowers on cane groundless, but that the flowering crop is usually superior, and gives better yields with a lower glucose ratio and a higher purity coefficient.

Ratooning

The new imported varieties of Coimbatore canes lend themselves very favourably to ratooning, a practice which is now becoming common with the gradual disappearance of the old local varieties for which this practice was not suitable and seldom practised except in years of fodder scarcity when the entire cane crop was needed for feeding animals.

It was generally observed that yields from the local varieties ratoon crop, were not equal to those from the parent crops, while the reverse generally held good with Coimbatore varieties.

Ratooning, therefore, offers certain advantages to the zamindars, as the usual operations necessary, prior to sowing canes are eliminated, and there is a saving of the cost of seed. Furthermore the ratoon crop ripens earlier, and owing to its well established root system it is better able to withstand unfavourable weather conditions.

Such a combination of advantages is, therefore, rendering the practice increasingly popular, and it was noticed in the early years of the survey that the zamindars in the Karnal and Rohtak districts were rapidly adopting it. In the Bets of Beas the practice has also become common, and as conditions in this locality are very favourable for a *barani* crop the local zamindar prefers a ratoon to a plant crop; in fact in the riverian tracts of the Beas, ratoon crops ten years old have been seen.

The general popular belief is that the yield increases for two or three years, remains stationary for an equal period and then begins to decline. It is replaced by a plant crop when the cultivators find it no longer pays.

Experiments carried out at the Jullundur Agricultural Station, for a number of years have given results shown in Table LVI.

These figures show that in this case, Co. 213 whose yields steadily declined was less suited for ratooning than Co. 223. Co. 205 is considered to be the type most suitable for ratooning.

Observations made at Miani Afghanan (Hoshiarpur) in two successive seasons showed that both Co. 205 and Co. 285 were suitable for ratooning as is indicated in Table LVII.

The ratoon crop from both varieties is seen to have given higher yields than the original plant crop and the quality was also maintained.

The practice, however, presents one serious disadvantage in as much as the presence of the crop on the land throughout the year offers conditions of food and shelter favourable for the development of the numerous pests which prey on the crop.

Consequently if the cane crop of the Punjab is not to suffer undue damage on this account, extensive ratooning is not to be recommended without adequate measures of insect control.

Fibre

Fibre is an important constituent of the cane plant, as it provides the framework with texture and support, without which it could not stand.

Lodging is one of the factors which greatly affects the tonnage and quality of cane, with larger applications of manure, better irrigation facilities, and more up-to-date methods of cultivation, heavier crops are now obtained and a cane with a low fibre content will lodge at a much earlier stage of vegetative growth than one not so handicapped.

Co. 205 contains about 18·5 per cent of fibre and is hence capable of withstanding drought and rain better than most other varieties, and this helps to explain why it is such a heavy yielder.

Similarly Co. 285 with a fibre content varying from 16·5 per cent in Jullundur to 17·7 per cent in Gurdaspur, is well suited to a wide range of differences of climate and conditions of cultivation, while the high fibre content of Katha may explain its survival as one of the hardiest of the indigenous varieties.

Such canes are also appreciated by the Zaminder as they afford him an adequate supply of fuel for *gur* making. Table LVIII gives the percentages of fibre in some canes grown at Jullundur and Gurdaspur.

It is noteworthy that most of the local varieties have a high fibre content, a fact noted by Barnes (*loc. cit.*) who gave the following figures :—

Variety	Per cent fibre
Dhaulu	20·60
Katha	23·57
Kansar	18·33
Teru	19·39
Kahu	13·54
Ponda	10·60

(The last two are soft chewing canes and are chiefly cultivated near towns.)

As explained above a high fibre content has advantages, but is not in itself a desirable feature.

Most of the Java canes show a fibre content of only about 12 per cent, and it is evident that if better methods of cultivation and pest control were possible, and lodging could be avoided a low fibre cane would be preferable to one with a high fibre content.

Effect on the cane crop of withholding water from October onwards

In the season of 1932-33, which as we have previously seen was characterised by prolonged frost in December and January, it was decided to study the effect on the cane crop of withholding water during October and subsequent months.

For this purpose separate fields of cane each under one variety were selected and divided into three portions each about 50 feet wide and 50 feet long. The two outer portions received normal irrigation while the central one was left without irrigation, and in order to eliminate the effect of lateral seepage strips from 10-15 ft. wide on either side of the central portion were omitted from the calculations.

The varieties selected for test were Co. 213 and Co. 223 at the Jullundur farm and at Jallowal in the Jullundur tehsil, Co. 213 at Baiyanpur near Sonepat and Co. 223 at the Hansi Agricultural Farm.

The results of the analyses from these trials are shown in Table LIX as the averages of figures obtained during the period of ripeness, and the following conclusions are deduced from them as the result of withholding irrigation :—

(*a*) The yield of both Co. 213 and Co. 223 was adversely affected.

(*b*) The quality of cane was not materially affected.

(*c*) The percentage of juice showed no significant differences.

(*d*) The fibre and glucose percentages were slightly less in canes receiving less water.

(*e*) The canes remained shorter but became thicker.

Effect of artificial manures on sugarcane

Although the survey under discussion was not primarily concerned with the effect of manures on cane, some specific observations were made in 1930-31, at the Gurdaspur Agricultural Station to ascertain the relative effects of certain artificial manures on the analytical data obtained from Co. 223.

Sugarcane is an exhaustive crop and in any intensive system of rotations in which it is included, the necessity for adequate manuring is an axiom of good husbandry.

While therefore the problem of the effects of manures on cane and the economic aspects of their employment forms by itself a separate enquiry, certain data is given here as illustrating the results obtained on Co. 223 with the following artificial manures :—

Manure	Rate of application
Potassium nitrate	2 Mds. per acre
Super-phosphate	1½ Mds. per acre
Ammonium sulphate	1½ Mds. per acre
Ammonium phosphate	1½ Mds. per acre

Half the dose was applied on May 15th, 1930, and the other half on June 24, 1930, just before the rains.

Table LX shows the averages of data obtained from the trial crops analysed during the 1930-31 season.

The highest yields were obtained from canes manured with potassium nitrate and ammonium sulphate respectively.

It was intended to repeat these trials in the following year, but it became necessary to ratoon the experimental crops owing to lack of water, and so the ratoon crops were subsequently analysed with the results shown below, which correspond with Table LX :—

Showing effect of artificial manure on Co. 223 ratoon crop

Potassium nitrate	15·3	1·41	0·064	1·62	62·0	9·2	0·42	10·6
Super-phosphate	12·7	1·16	0·052	1·33	60·9	9·1	0·41	10·5
Ammonium sulphate	16·1	1·48	0·068	1·71	60·8	9·2	0·42	10·6
Ammonium phosphate	12·8	1·18	0·047	1·33	60·0	9·2	0·37	10·4
Control	12·3	1·13	0·049	1·28	60·0	9·2	0·40	10·4

In the year 1932-33, these trials were repeated using Co. 285 as the test plant crop and the results are given in Table LXI.

The results of the three years' observations indicate that the canes in question respond best to potassium nitrate and ammonium sulphate in regard to yields Ammonium sulphate and Ammonium phosphate, however, delayed ripening to some extent, while potassium nitrate and super-phosphate had no such effect.

Furthermore the artificials employed only affected the tonnage yields and were without influence either on the quality or quantity of juice obtained (apart from the ash content) or on the percentage of fibre in the cane. Another interesting feature was the fact that canes treated with potassium nitrate showed a greater resistence to low temperatures; their juice showed a lower freezing point and a higher ash content, while the fibre content was unaffected.

These results have been confirmed by manurial trials carried at the Hansi Agricultural Farm during the last two seasons of the survey, when ammonium sulphate and ammonium phosphate, although showing increased yields, caused no difference in the quality of the juice.

Summary

1. The survey here described was conducted for four years in the Rohtak-Karnal area and for six years in the Jullundur and Gurdaspur Circles.

2. The Coimbatore varieties have on the whole given better yields of cane and sucrose per acre in all the centres surveyed.

The average figures for all localities are shown in Tables LXII and LXIV.

The sugar industry in the Punjab must depend for its success very largely on these new varieties, although Suretha among the indigenous canes was still a very successful cane in the Southern Punjab.

3. The quality of the Coimbatore canes has shown a steady improvement since 1928-29, mainly due to the introduction of new and better types.

Until 1931-32, Co. 213, Co. 285 and 290 were undoubtedly the best canes found and to a large degree the improvement in sugarcane production can be traced to them. Co. 281 and Co. 285 are the next best. During the last season, however, four new types of cane, Co. 300, Co. 301, Co. 312, and Co. 313, gave still better

results. The first of these, in addition to its other qualities, was also immune to attack by Pyrilla, due perhaps to the presence of a growth of hairy bristles on the under surface of its leaves. Both in regard to yield and quality, Co. 300 has proved to be the best cane in the Gurdaspur Circle, while the last three types have so far only been introduced in the Jullundur area (Tables LXII-LXIV and XVI).

4. Average figures for the yields of cane per acre during the period of the survey show that, of the localities surveyed, the Southern Punjab is, taking all factors into consideration, the best suited for sugarcane production.

On the other hand if we consider the amount of sucrose obtained from a normal crop per acre the Jullundur and Hoshiarpur districts, in spite of a somewhat lower tonnage yield, have given the best results. It must be remembered, however, that some of the new Coimbatore varieties which are responsible for the improvement of cane in the latter area were not included in the survey of the former tract, which terminated in 1931. Further analytical investigation may show that these newest varieties are doing still better in the more suitable south-eastern portion of the Province.

5. With the introduction of new varieties the period of optimum ripeness, during which cane is available for crushing has been considerably extended, although it varies in different localities (Tables VIII, XVII and XXVI). The average period as determined during the survey was 118 days for Rohtak and Karnal, 130 for Gurdaspur and 135 for Jullundur and Hoshiarpur, although some slight modification of these figures may be necessary, owing to the earlier termination of the survey in the Rohtak and Karnal area.

Most of the Coimbatore varieties do not ripen before the first week of December, so in order to increase the length of the crushing season, or for early crushing, local varieties such as Katha, Dhaulu and Lalri, will still be required. It is therefore very desirable that efforts be made to produce new Coimbatore varieties which, in addition to their existing desirable qualities, will also be early ripeners, since in view of the usual agricultural practice followed in the Punjab any lengthening of the crushing season beyond the end of March is not desirable.

No single variety can, however, be expected to have a period of optimum ripeness of more than, if as much as, 100 days, and so a judicious blending of types with graded maturity is necessary for a long crushing period.

6. It is not possible to form any systematic and exact estimate of the extent of damage caused to the cane crop by frost, insect pests and disease, since the incidence and intensity of these factors vary widely from year to year.

Local varieties such as Katha and Lalri may, however, be classified as semi-resistant to frost, Co. 281 and Co. 285, as more or less resistant, while Co. 223 is usually damaged.

It is too early yet to offer a decided opinion on the reaction of Co. 300, Co. 301, Co. 312, and Co. 313 to frost as the observations so far made require to be confirmed in the future.

The very succulent leaves and low fibre content of Co. 223, and Co. 290, render these types particularly susceptible to injury by insects and disease.

7. The flowering of canes, which has been observed to occur only in the case of Coimbatore varieties, marks the end of vegetative growth and does not indicate any deterioration in quality as has been supposed by some cultivators ; on the other hand canes in flower usually indicate heavier crops than do flowerless canes. It has been observed that scarcity of rainfall during September, October, November and December is conducive to conditions favourable to flowering.

8. Coimbatore varieties are well adapted for ratooning and therefore with their introduction the practice of ratooning is becoming popular, specially in the Bet or riverian tracts of the Province. The presence of sugarcane in the soil throughout the year, however, supplies a steady source of food to insects and pests common to this crop. A close watch on a ratoon crop is, therefore, necessary if the multiplication of these diseases and pests, to the great risk of injury to neighbouring healthy crops is to be avoided.

9. Sugarcane, particularly the new varieties, shows, under normal conditions, a definite response to the application of artificial manures, provided other features, such as irrigation, cultivation, etc., are attended to.

Of the manures tried ammonium sulphate and ammonium phosphate appear to give the best results as regards yields, although there is no appreciable difference in the quality of the juice obtained from manured and unmanured crops of the same variety.

10. Withholding irrigation from October onwards results in decreasing the yield of cane per acre, although the composition of the juice is not affected to any appreciable extent.

REFERENCE

Barnes, J. H. (1918). *Agric. Res. Inst., Pusa Bull.* No. 69.

Tables I to LXV

Figures for total solids, sucrose and glucose given in these tables, were originally determined in juice and then calculated on cane on the basis of extractable juice. Thus they do not represent the absolute quantity of these constituents present in the cane. This fact should also be remembered in considering the outturn of these substances per acre or per ton of cane.

During the survey over 4,500 analyses were carried out and as it is not convenient to present the results of the whole of these, data presented in Tables I to XXVI and some of the other tables are based on the averages of the figures for the different constituents of canes during the period of " optimum ripeness ". Each variety of cane was analysed at fortnightly intervals, generally from the beginning of November to the middle of April.

TABLE I

Average composition of Lalri during period of ripeness (Karnal and Rohtak districts)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
1927-28 .	Shamaspur . . .	28·50	48·24	8·90	7·62	0·035	0·46	85·6
	Kalupur . . .	34·86	44·22	8·52	7·39	0·039	0·52	87·8
	Sheikhupura . .	23·77	48·87	10·00	8·42	0·025	0·30	84·3
Average for the year.	..	29·04	47·11	9·14	7·81	0·033	0·42	85·4
1928-29 .	Charao . . .	14·17	44·51	8·95	7·17	0·21	2·30	80·1
	Budhshyam . . .	22·48	43·37	8·57	7·20	0·11	1·52	84·0
	Shamaspur . . .	21·60	44·60	8·96	7·53	0·13	1·80	84·0
	Sonepat . . .	24·45	46·60	9·35	7·62	0·14	1·74	81·6
Average for the year.	..	20·68	44·77	8·95	7·38	0·15	2·00	82·5
1929-30 .	Sonepat . . .	14·27	55·20	10·89	9·33	0·06	0·70	85·6
	Machhroli . . .	19·98	61·40	10·42	8·01	0·12	1·45	76·9
	Jharautha . . .	12·85	57·19	11·01	9·14	0·11	1·26	83·1
	Shamaspur . . .	15·70	56·46	11·05	8·91	0·17	1·92	80·6
Average for the year.	..	15·56	57·56	10·84	8·85	0·11	1·24	81·6
1930-31 .	Mohammadabad . .	16·20	55·30	10·42	7·56	0·19	2·22	82·0
	Jatheri . . .	16·10	56·30	9·85	7·95	0·17	2·15	80·7
	Lehrara . . .	17·40	57·40	9·27	7·06	0·27	3·83	76·1
	Jaji	15·90	58·40	9·65	7·38	0·19	2·54	76·2
Average for the year	..	16·40	56·85	9·75	7·74	0·21	2·70	79·4
Average for four years	..	20·42	51·57	9·67	7·94	0·12	1·57	82·1
Average yield for the last three years	..	17·55	..	..	..	..	..	..

TABLE II

Average composition of Suretha and Dhaulu during period of ripeness (Karnal and Rohtak districts)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
			Suretha					
1927-28 . . .	Shamaspur .	50·15	52·22	10·95	9·47	0·05	0·49	86·4
	Kalupur . .	32·80	53·00	9·68	7·87	0·11	1·44	81·3
	Sheikhupura .	45·40	56·40	11·02	9·22	0·04	0·39	83·5
Average for the year	..	**42·78**	**53·87**	**10·55**	**8·85**	**0·07**	**0·73**	**83·9**
1928-29 . . .	Chirao . .	18·78	45·26	8·25	6·83	0·24	2·90	78·6
	Budhshiam .	32·25	48·57	9·67	8·23	0·09	1·16	85·8
	Shamaspur .	23·91	52·90	10·70	8·57	0·17	2·07	84·4
	Sonepat . .	24·15	51·14	10·00	8·35	0·25	2·90	82·4
Average for the year	..	**24·77**	**49·44**	**9·66**	**8·00**	**0·19**	**2·10**	**82·8**
1929-30 . . .	Sonepat . .	26·31	60·69	11·37	9·41	0·10	1·02	82·8
	Machhrauli .	29·07	68·36	10·23	7·71	0·16	2·05	75·4
	Shamaspur .	16·62	60·33	11·67	10·02	0·22	2·23	85·9
Average for the year	..	**24·00**	**63·13**	**11·09**	**9·05**	**0·16**	**1·80**	**81·6**

TABLE II—*contd.*

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
1930-31 . . .	Fatehpur .	22·10	66·40	10·89	9·01	0·23	2·56	82·7
	Jatheri . .	18·90	61·50	12·27	10·22	0·37	3·64	83·3
	Lehrara (Pyrilla and frost) affected	22·90	65·00	7·56	4·61	0·51	0·23	67·3
	Phusgarh .	19·60	52·10	9·79	7·40	0·23	3·10	75·6
	Jaji . .	14·50	57·60	10·21	8·27	0·20	2·41	80·9
Average for the year	..	**19·60**	**58·52**	**10·14**	**7·90**	**0·31**	**3·90**	**77·9**
Average of four years	..	**27·79**	**56·49**	**10·36**	**8·45**	**0·18**	**2·14**	**81·6**
Average yield for the last three years	..	**22·79**	..	..	..	..	..	..
			Dhaulu					
1927-28 . . .	Chhaparya .	26·0	57·25	10·80	9·05	0·04	0·48	83·8
1928-29 . . .	Khara (*barani*).	25·18	45·10	8·14	7·22	0·14	1·75	89·1
Average of two years	..	**25·59**	**48·18**	**9·47**	**8·14**	**0·09**	**1·10**	**86·0**

TABLE III

Average composition of Co. 205 during period of ripeness (Karnal and Rohtak districts)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
1927-28	Shamaspur	25·40	52·97	10·30	8·85	0·09	0·96	86·0
	Kalupur	36·30	52·67	10·29	8·65	0·18	1·31	84·0
	Jaurasi	23·10	51·10	10·49	9·32	0·05	0·58	90·0
	I. C. B. Farm	28·60	48·49	9·10	7·90	0·10	1·39	86·8
Average for the year	..	**28·30**	**51·31**	**10·06**	**8·68**	**0·11**	**1·20**	**83·5**
1928-29	Chirao	27·61	49·03	9·52	8·21	0·28	3·32	86·2
	Budhshiam	31·55	46·70	8·90	7·52	0·27	3·68	83·3
	Shamaspur	26·83	52·00	8·90	7·09	0·15	2·20	80·0
	Sonepat	21·75	51·86	10·00	7·80	0·71	9·20	78·3
Average for the year	..	**26·93**	**49·90**	**9·33**	**7·65**	**0·35**	**4·60**	**82·0**
1929-30	Sonepat	23·26	57·00	10·24	8·61	0·12	1·40	84·1
	Machhrauli	29·54	60·62	11·05	9·25	0·22	2·35	83·7
	Jharauntha	17·71	59·85	11·02	9·28	0·21	2·31	84·2
	Shamaspur	20·67	60·24	11·37	9·54	0·19	1·96	84·0
Average for the year	..	**22·80**	**59·43**	**10·92**	**9·18**	**0·18**	**1·96**	**82·2**
1930-31	Mohammadabad	18·60	52·80	10·08	8·77	0·21	2·43	86·4
	Lehrara	15·80	64·30	9·75	7·30	0·69	9·39	74·8
	Jaji	21·40	57·50	10·64	9·03	0·34	3·78	85·0
Average for the year	..	**18·95**	**58·21**	**10·15**	**8·37**	**0·41**	**4·90**	**82·5**
Average of four years	..	**24·25**	**54·71**	**10·16**	**8·47**	**0·35**	**4·13**	**82·6**
Average yield for the last three years	..	**22·90**	..	..	..	..	..	..

TABLE IV

Average composition of Co. 213 during period of ripeness (Karnal and Rohtak districts)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
1927-28 . . .	Machhrauli .	40·10	59·1	11·82	10·80	0·03	0·24	91·4
1928-29 . . .	I. C. B. Farm .	30·22	52·8	9·19	7·35	0·16	2·30	80·2
	Budhshiam .	29·72	55·6	9·77	8·51	0·22	2·81	85·0
	Sonepat . .	25·44	56·3	10·17	8·69	0·22	2·51	84·4
Average for the year	..	**28·46**	**54·9**	**9·71**	**8·18**	**0·20**	**2·45**	**84·2**
1929-30 . . .	Sonepat . .	20·03	59·8	10·95	9·69	0·06	0·58	88·4
	Machhrauli .	28·61	67·0	12·53	11·09	0·09	0·81	88·5
Average for the year	..	**24·32**	**63·4**	**11·74**	**10·39**	**0·08**	**0·77**	**88·5**
1930-31 . . .	Fatehpur .	23·10	61·4	10·14	8·57	0·34	4·05	84·6
	Jatheri . .	18·40	63·4	10·70	9·25	0·30	3·31	86·4
	Phusgarh .	22·60	64·9	10·24	8·03	0·31	3·76	78·7
	Jaji . .	23·90	63·4	9·40	7·80	0·48	6·25	82·8
Average for the year	..	**22·00**	**63·3**	**10·12**	**8·41**	**0·36**	**4·30**	**83·1**
Average of four years	..	**28·72**	**60·2**	**10·86**	**9·45**	**0·17**	**1·80**	**87·1**
Average yield for the last three years	..	**24·93**	..	..	..	..	..	..

TABLE V

Average composition of Co. 223, Co. 210 and Co. 214 during period of ripeness (Karnal and Rohtak districts)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity coefficient
			Juice	Total solids	Sucrose	Glucose		
		Co. 223						
1927-28 . . .	Shamaspur .	26·90	59·47	11·48	10·40	0·08	0·79	90·6
1928-29 . . .	I. C. B. Farm .	39·32	48·60	8·50	6·85	0·24	3·50	81·5
	Budhshiam .	29·12	51·55	8·58	7·10	0·27	3·81	82·6
	Shamaspur .	25·55	52·84	9·40	8·06	0·13	1·66	85·7
	Sonepat . .	28·26	49·03	8·25	7·50	0·08	1·08	84·8
Average for the year	..	**30·56**	**50·50**	**8·68**	**7·38**	**0·18**	**2·43**	**85·2**
1929-30 . . .	Sonepat . .	28·42	62·31	11·30	10·04	0·13	1·26	88·8
	Jharauthi .	17·76	64·05	11·91	10·29	0·20	1·97	86·4
	Shamaspur .	24·86	62·40	10·93	9·57	0·19	1·97	87·4
Average for the year	..	**23·68**	**62·92**	**11·38**	**9·97**	**0·17**	**1·71**	**87·6**
Average of three years	..	**27·05**	**57·63**	**11·18**	**9·25**	**0·14**	**1·52**	**82·8**
Average yield for the last two years	..	**27·12**	..	..	..	..	..	..
		Co. 210						
1927-28 . .	I. C. B. Farm .	19·96	52·51	9·26	8·06	0·06	0·78	87·0
		Co. 214						
1927-28 . .	I. C. B. Farm .	18·80	53·83	9·82	8·45	0·05	0·64	86·0

TABLE VI

Amount of juice and sucrose, etc., per acre (in tons) and per ton of cane (Karnal and Rohtak districts)

Variety	Year	Weight per acre				Weight per ton of cane		
		Stripped canes	Juice	Total solids	Sucrose	Juice	Total solids	Sucrose
Lalri . . .	1927-28 . .	29·04	13·70	2·65	2·27	0·471	0·091	0·078
	1928-29 . .	20·68	9·15	1·81	1·51	0·448	0·090	0·074
	1929-30 . .	15·56	8·96	1·68	1·38	0·576	0·108	0·089
	1930-31 . .	16·40	9·32	1·61	1·26	0·569	0·098	0·077
	Average . .	**20·42**	**10·32**	**1·94**	**1·58**	**0·516**	**0·097**	**0·079**
Suretha . . .	1927-28 . .	42·78	23·10	4·54	3·82	0·539	0·106	0·089
	1928-29 . .	24·77	12·22	2·40	1·98	0·494	0·097	0·080
	1929-30 . .	24·00	15·18	2·66	2·18	0·631	0·111	0·091
	1930-31 . .	19·60	11·47	1·98	1·55	0·585	0·101	0·079
	Average .	**27·79**	**15·70**	**2·89**	**2·36**	**0·565**	**0·104**	**0·085**
Dhaulu . . .	1927-28 . .	26·00	13·35	2·81	2·36	0·513	0·108	0·091
	1928-29 . .	25·18	11·35	2·04	1·81	0·451	0·081	0·072
	Average .	**25·59**	**12·35**	**2·43**	**2·08**	**0·482**	**0·095**	**0·081**

Co. 285 . .	1927-28 . .	28·30	14·56	2·94	2·46	0·513	0·104	0·087
	1928-29 . .	26·93	13·45	2·51	2·08	0·499	0·093	0·077
	1929-30 . .	22·80	13·54	2·53	2·10	0·594	0·111	0·092
	1930-31 . .	18·95	8·29	1·93	1·59	0·437	0·102	0·084
	Average .	**24·25**	**12·50**	**2·62**	**2·02**	**0·511**	**0·103**	**0·085**
Co. 213 . . .	1927-28 . .	40·10	24·95	4·78	4·38	0·591	0·118	0·108
	1928-29 . .	28·46	15·60	2·62	2·43	0·549	0·092	0·082
	1929-30 . .	24·32	15·50	2·85	2·53	0·634	0·117	0·104
	1930-31 . .	22·00	13·93	2·22	1·85	0·633	0·101	0·084
	Average .	**28·72**	**17·30**	**3·13**	**2·74**	**0·602**	**0·109**	**0·095**
Co. 223 . . .	1927-28 . .	26·90	16·00	3·10	2·80	0·595	0·115	0·104
	1928-29 . .	30·56	15·43	2·76	2·26	0·505	0·087	0·074
	1929-30 . .	23·68	16·55	3·00	2·58	0·629	0·114	0·098
	Average .	**27·05**	**15·99**	**2·95**	**2·55**	**0·576**	**0·105**	**0·093**

TABLE VII

Composition of juice from different varieties of cane—average of four years (Karnal and Rohtak districts)

Variety of cane	Weight of stripped cane in tons per acre	Per cent on cane of				Glucose ratio	Purity coefficient
		Juice	Total solids	Sucrose	Glucose		
Lalri	20·42	51·57	9·67	7·94	0·10	1·51	82·1
Suretha	27·79	56·49	10·36	8·45	0·18	2·10	81·6
Dhaulu	25·59	48·18	9·47	8·14	0·09	1·11	86·0
Co. 205	24·25	51·07	10·26	8·47	0·35	4·13	82·6
Co. 213	28·72	60·18	10·86	9·45	0·17	1·80	87·0
Co. 223	27·05	57·63	11·18	9·25	0·14	1·51	82·7
Local (Average)	**24·60**	**52·08**	**9·83**	**8·18**	**0·12**	**1·47**	**83·2**
Coimbatore (Average) . . .	**26·67**	**56·29**	**10·77**	**9·06**	**0·22**	**2·40**	**84·1**

TABLE VIII

Period of ripeness for different varieties of cane (Karnal and Rohtak districts)

Year	Varieties of canes					
	Lalri	Suretha	Dhaulu	Co. 205	Co. 213	Co. 223
1927-28 . . .	15/12 to 10/2	3/2 to 21/3	30/12 to 10/4	1/2 to 31/3	15/2 to 1/4	20/2 to 1/4
1928-29 . . .	1/1 to 15/3	15/1 to 15/3	1/1 to 7/2	15/1 to 31/3	15/1 to 15/3	15/1 to 15/3
1929-30 . . .	25/11 to 15/3	25/11 to 31/3	..	10/1 to 31/3	10/1 to 31/3	1/1 to 31/3
1930-31 . . .	23/11 to 1/2	25/1 to 3/4	..	15/12 to 9/4	20/2 to 5/4	20/12 to 15/3
Average period of optimum ripeness	9/12 to 5/3	6/12 to 25/3	31/12 to 21/3	10/1 to 2/4	30/1 to 28/3	14/1 to 23/3

Generally speaking the crushing season may well start with the beginning of December and last till the end of March, *i.e.*, about 120 days.

TABLE IX

Average composition of Katha, Dhaulu and Yuba during period of ripeness (Jullundur and Hoshiarpur districts)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity coefficient
			Juice	Total solids	Sucrose	Glucose		
			Katha					
1927-28 . . .	Badala (Bajekhan's)	24·6	61·00	12·14	10·43	0·05	0·4	86·0
	Bhundian .	17·7	62·2	11·82	10·18	0·04	0·4	86·4
	Naugaja (Attar's)	20·3	62·3	11·95	10·22	0·14	1·4	85·5
	Sundarpur .	29·9	62·5	12·25	10·40	0·13	1·2	84·9
	Nagke . .	8·1	62·4	12·49	10·50	0·07	0·7	84·4
	Naugaja (Ishas Singh's)	26·1	61·4	11·91	10·40	0·06	0·6	87·3
	Badala (Jamilkhan)	35·7	60·7	11·72	10·10	0·07	0·7	86·0
	Jandusingha .	21·7	62·5	12·02	10·29	0·11	1·1	86·6
Average for the year	..	**23·0**	**61·9**	**12·04**	**10·32**	**0·08**	**0·8**	**85·7**

1928-29	Kundhalian .	10·7	55·6	10·12	8·40	0·17	2·0	83·0
	Khudda .	15·0	59·5	10·59	8·81	0·30	0·4	83·0
	Rasulpur .	10·3	59·4	10·22	8·61	0·23	3·0	84·0
	Bhundian .	20·3	61·8	10·26	8·49	0·23	3·0	83·0
	Dingrian .	11·7	55·8	10·49	8·76	0·17	2·0	84·0
	Badala Mansurpur	11·2	54·9	10·60	8·84	0·19	2·0	83·0
	Hussainpur .	12·1	55·1	9·53	7·66	0·39	5·0	80·0
	Ramowal (Nakodar)	10·3	57·0	10·06	9·12	0·07	0·7	82·0
	Dhaliwal .	11·0	57·2	9·44	7·66	0·31	4·0	81·0
	Sargondi .	8·9	54·9	10·04	8·62	0·08	1·0	86·0
	Jindowal .	11·5	55·7	10·92	9·36	0·11	1·0	86·0
Average for the year	..	**12·1**	**56·9**	**10·29**	**8·58**	**0·20**	**2·3**	**83·4**
1929-30	Jindowal .	11·5	51·6	9·90	8·20	0·15	2·0	83·0
	Mehli . .	14·7	53·6	10·80	9·30	0·07	1·0	87·0
	Mahl . .	14·9	54·2	10·80	9·00	0·11	1·0	83·0
	Dhaliwal .	16·3	52·8	10·20	8·50	0·16	2·0	83·0
Average for the year	..	**14·4**	**53·1**	**10·40**	**8·80**	**0·12**	**1·4**	**84·6**

TABLE IX—*contd.*

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity coefficient
			Juice	Total solids	Sucrose	Glucose		
1930-31.	Jabbowal .	12·6	54·1	10·40	8·30	0·13	2·0	80·0
	Sargondi .	12·1	55·7	10·70	8·80	0·34	4·0	82·0
	Dhaliwal .	10·5	54·1	10·00	8·00	0·34	4·0	80·0
	Jullundur .	13·6	58·4	11·00	9·00	0·15	2·0	81·0
	Bhundian .	15·6	58·7	11·30	9·40	0·16	2·0	83·0
	Khudda . .	15·5	57·8	9·90	8·30	0·45	5·0	84·0
	Jhingar Kalan	10·3	56·7	10·10	8·60	0·35	4·0	85·0
Average for the year	..	**12·9**	**56·4**	**10·50**	**8·60**	**0·27**	**3·1**	**81·9**
1931-32	Chuheki .	16·0	60·8	11·40	9·10	0·49	5·4	80·0
	Rehrum .	15·0	56·1	10·70	8·70	0·12	1·4	81·0
	Rasulpur .	18·5	55·5	10·80	9·10	0·18	2·0	84·0
	Jullundur A. S.*	14·9	57·1	10·90	8·20	0·65	7·9	75·0
Average for the year	..	**16·5**	**57·5**	**11·00**	**9·00**	**0·26**	**2·8**	**81·7**

1932-33	Jullundur A. S.*	15·7	57·1	10·90	8·80	0·21	2·4	81·0
Average of six years	..	15·8	57·2	10·86	9·02	0·19	2·1	83·1
Average yield for the last five years	..	14·3	..	..	..	..	..	..
			Dhaulu					
1927-28	Bal . .	24·60	62·60	10·49	9·02	0·06	0·6	86·0
			Yuba					
1927-28	Duleke . .	27·3	67·10	12·22	10·83	0·34	3·1	88·6

* Abnormal sample, figures not taken into calculation.

TABLE X

Average composition of Co. 205 during period of ripeness (Jullundur and Hoshiarpur districts)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity coefficient
			Juice	Total solids	Sucrose	Glucose		
1927-28	Kapurpind .	33·8	66·5	11·41	9·01	0·81	8·9	79·0
	Prabhunagar .	27·3	58·8	10·91	9·18	0·31	3·4	84·1
	Bhundian .	21·3	63·8	11·42	9·93	0·25	2·6	86·9
	Bariah . .	29·1	62·7	11·20	9·44	0·35	3·7	83·5
	Dhingrian .	46·5	68·2	11·77	9·86	0·41	4·2	83·8
	Jandusingha .	41·4	57·0	10·85	9·34	0·22	2·3	86·0
	Beaspind .	15·8	59·6	10·99	9·24	0·41	4·4	84·1
	Jullundur (*Chahi*)	13·5	61·3	12·66	9·86	0·10	1·0	78·0
	Jullundur (*Barani*)	7·7	59·0	12·21	9·66	0·14	1·4	79·0
Average for the year	..	26·3	61·9	11·49	9·50	0·33	3·5	82·7

1928-29	Nawanpind .	21·1	61·6	9·18	7·14	0·80	11·0	78·0
	Passi Bet .	17·2	62·8	9·86	7·85	0·69	9·0	79·0
	Passi Bet (Ratoon)	15·8	61·3	10·18	8·21	0·86	10·0	81·0
	Bhundian .	17·5	64·0	10·18	8·00	0·49	6·0	79·0
	Dhingrian .	14·8	61·6	9·30	7·70	0·67	9·0	82·0
	Hussainpur .	17·3	61·0	8·42	6·41	1·35	24·0	71·0
	Ramowal (Nakodar)	13·2	60·1	10·64	8·83	0·28	3·0	83·0
	Dhaliwal .	11·2	62·9	10·87	7·67	0·94	12·0	76·0
	Sargondi .	21·1	61·9	10·03	7·68	1·11	15·0	76·0
	Kothran .	18·6	57·6	11·12	9·04	0·51	6·0	81·0
	Jindowal .	18·0	59·5	10·95	9·16	0·36	4·0	84·0
Average for the year	..	16·9	61·3	10·07	7·97	0·73	9·2	79·2
1929-30	Jindowal .	24·4	58·5	11·30	9·60	0·26	3·0	85·0
	Mehli . .	22·8	59·1	11·70	9·80	0·19	2·0	84·0
	Sargondi .	20·7	54·4	11·30	9·60	0·40	4·0	85·0
	Dhaliwal .	19·2	57·5	10·90	8·80	0·43	5·0	81·0
Average for the year	..	21·8	57·4	11·30	9·50	0·32	3·4	84·1

TABLE X—*contd.*

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity coefficient
			Juice	Total solids	Sucrose	Glucose		
1930-31	Jabbowal .	17·2	61·1	11·40	9·60	0·62	7·0	84·0
	Sargondi .	24·1	62·3	11·80	9·50	0·51	5·0	81·0
	Dhaliwal .	21·2	59·7	11·40	9·10	0·65	7·0	80·0
	Bhundian .	27·9	63·2	11·40	9·40	0·76	8·0	83·0
	Jhingar Kalan	21·0	61·2	9·80	7·50	1·20	16·0	77·0
Average for the year	..	22·3	61·5	11·20	9·00	0·75	8·3	80·3
1931-32	Jabbowal .	22·1	58·5	11·60	9·30	0·74	7·9	80·0
	Chuheki .	24·9	60·6	11·70	9·20	0·91	9·9	79·0
	Khurdpur .	31·1	61·5	11·10	8·70	0·79	9·1	78·0
	Miani Afghana	13·6	59·0	10·80	8·40	0·63	7·5	78·0
	Miani Afghana (Ratoon)	27·7	60·0	11·00	8·90	0·55	6·2	81·0
Average for the year	..	23·9	59·9	11·24	8·90	0·72	8·1	79·2

1932-33	Miani Afghana	17·2	59·1	11·30	9·10	0·68	7·5	81·0
	Khurdpur	25·8	61·7	11·80	9·50	0·66	6·9	80·0
Average for the year	..	21·5	60·4	11·55	9·30	0·67	7·2	81·0
Average for six years	..	22·1	60·4	11·14	9·03	0·59	6·5	81·1
Average yield for the last five years	..	21·3	..	..	..	..	..	..

TABLE XI

Average composition of Co. 213 during period of ripeness (Jullundur and Hoshiarpur districts)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
1928-29 . . .	Badala Mansurpur	7·7	62·8	10·30	8·79	0·41	5·0	86·0
	Hussainpur (*Pyrilla*)	12·4	64·1	8·72	6·54	1·15	17·0	74·0
	Ramowal .	9·8	61·5	11·07	9·59	0·92	1·0	87·0
	Dhaliwal .	16·2	63·4	10·08	8·31	0·32	4·0	82·0
	Sargondi (*Pyrilla*)	20·7	65·1	8·79	6·64	1·04	16·0	76·0
	Banga . .	21·1	64·8	12·44	10·67	0·17	2·0	85·0
	Karyam . .	17·9	61·0	12·02	10·37	0·09	1·0	87·0
Average for the year	..	**15·1**	**63·3**	**10·49**	**8·40**	**0·59**	**7·0**	**80·1**
1929-30 . . .	Jindowal .	21·5	61·3	11·00	9·50	0·26	3·0	86·0
	Banga . .	35·2	64·1	10·20	8·50	0·26	3·0	83·0
	Khothran .	10·4	55·2	10·20	8·90	0·07	1·0	87·0
	Sargondi .	18·9	59·3	10·60	9·30	0·31	3·0	88·0

	Dhaliwal .	22·6	63·0	11·50	10·20	0·16	2·0	88·0
Average for the year	..	**21·7**	**60·6**	**10·70**	**9·30**	**0·21**	**2·3**	**86·9**
1930-31 . . .	Jabbowal .	20·3	67·5	12·80	11·30	0·28	3·0	89·0
	Sargondi .	20·7	68·5	12·20	10·60	0·34	3·0	87·0
	Dhaliwal .	17·2	63·4	12·50	11·00	0·12	1·0	87·0
	Jullundur A. S.	20·6	64·8	12·50	10·90	0·18	2·0	87·0
	Bhundian .	18·5	69·5	11·70	9·80	0·35	4·0	83·0
Average for the year	..	**19·5**	**67·1**	**12·30**	**10·70**	**0·25**	**2·3**	**87·0**
1931-32 . . .	Jabbowal .	34·6	66·2	12·80	10·80	0·49	4·5	84·0
	Sargondi .	31·1	65·2	12·60	10·80	0·51	4·7	86·0
	Khurdpur .	38·6	66·9	13·00	11·30	0·33	2·9	87·0
	Jullundur A. S.	16·5	67·8	12·50	10·30	0·37	3·7	83·0
Average for the year	..	**30·2**	**66·5**	**12·70**	**10·80**	**0·43**	**4·0**	**85·0**
1932-33 . . .	Mehli . .	17·9	65·3	12·90	11·40	0·09	0·8	89·0
	Nakodar .	19·4	67·7	12·30	10·30	0·17	1·6	85·0
	Khurdpur .	17·2	66·8	12·20	10·80	0·17	1·6	89·0
	ullundur A. S.	34·9	67·1	12·20	10·20	0·27	2·7	84·0
Average for the year	..	**22·4**	**66·7**	**12·40**	**10·70**	**0·18**	**1·7**	**86·3**
Average of five years	..	**21·8**	**64·8**	**11·72**	**9·98**	**0·33**	**3·3**	**85·2**

TABLE XII

Average composition of Co. 223 during period of ripeness (Jullundur and Hoshiarpur districts)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
1927-28 . . .	Jullundur Farm	21·00	67·6	12·22	10·51	0·12	1·2	86·2
	Badala (Bajekhan).	33·04	66·5	11·48	10·35	0·17	1·6	90·0
	Badala (Jamilkhan).	39·56	67·8	11·56	10·40	0·20	1·9	90·0
	Bhundian .	28·27	66·5	11·41	10·08	0·19	1·9	88·3
Average for the year	..	**30·50**	**67·1**	**11·68**	**10·33**	**0·17**	**1·6**	**88·4**
1928-29 . . .	Nawanpind .	23·00	64·1	9·29	7·88	0·39	5·0	85·0
	Passi Bet .	16·80	65·3	9·34	7·71	0·59	8·0	82·0
	Rasulpur .	18·00	65·8	9·74	8·03	0·59	8·0	82·0
	Bhundian .	22·70	65·8	10·00	7·83	0·41	6·0	78·0
	Badala Mansurpur.	15·40	59·5	9·76	8·51	0·39	5·0	87·0
	Hussainpur .	14·30	63·7	8·28	5·99	1·27	21·0	72·0
	Ramowal (Nakodar).	8·30	60·0	10·50	9·18	0·08	1·0	88·0

	Dhaliwal .	15·40	62·1	9·31	6·89	0·69	10·0	74·0
	Sargondi .	21·20	62·4	8·80	7·05	0·93	13·0	80·0
	Kothran . .	25·10	65·5	9·83	7·86	0·59	8·0	80·0
	Jindowal .	8·60	60·7	10·32	9·23	0·29	3·0	85·0
Average for the year	..	**15·40**	**63·2**	**9·56**	**7·83**	**0·56**	**7·2**	**81·9**
1929-30 . . .	Jindowal .	24·50	59·7	10·40	9·00	0·27	3·0	86·0
	Mehli . .	20·40	57·7	10·30	8·90	0·15	2·0	86·0
	Mehli (*Pyrilla* affected).	21·20	57·7	10·20	8·90	0·17	2·0	87·0
	Sargondi .	19·10	55·0	10·10	9·20	0·24	3·0	91·0
	Dhaliwal .	20·20	61·0	11·40	10·20	0·19	2·0	89·0
Average for the year	..	**21·10**	**58·2**	**10·50**	**9·20**	**0·20**	**2·2**	**87·6**
1930-31 . . .	Jabbowal .	21·20	65·6	11·20	9·50	0·49	5·0	85·0
	Sargondi .	24·30	66·4	11·40	9·80	0·40	4·0	86.0
	Dhaliwal .	17·90	64·6	11·50	10·00	0·34	3·0	87·0
	Jullundur A. S.	24·60	67·2	11·30	9·60	0·62	7·0	85·0
	Bhundian .	22·10	65·6	11·40	9·50	0·54	6·0	84·0
	Rasulpur .	23·10	66·1	10·00	7·90	0·76	10·0	80·0
Average for the year	..	**22·20**	**65·9**	**11·10**	**9·40**	**0·53**	**5·6**	**84·7**

TABLE XII—*contd.*

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
1931-32 . . .	Sargondi .	27·00	63·7	11·70	9·80	0·65	6·6	82·0
	Mohanwal .	22·60	64·3	11·70	10·20	0·56	5·5	87·0
	Rasulpur .	30·90	62·1	11·40	9·80	0·51	5·2	85·0
	Jullundur A.S.	22·80	64·0	11·50	8·50	0·51	5·9	81·0
Average for the year	..	26·83	63·4	11·60	9·93	0·57	5·7	85·6
1932-33 . . .	Mehli . .	16·10	62·5	11·80	10·40	0·27	2·5	88·0
	Nakodar .	16·20	66·7	11·50	9·60	0·41	4·3	83·0
Average for the year	..	16·15	64·6	11·65	10·00	0·34	3·4	85·8
Average for six years	..	22·03	63·7	11·02	9·45	0·40	4·3	85·8
Average yield for the last 5 years	..	20·34	..	..	..	..	..	..

TABLE XIII

Average composition of Co. 281 during period of ripeness (Jullundur and Hoshiarpur districts)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
1931-32 . . .	1. Jabbowal .	19·7	66·7	13·0	11·7	0·19	1·6	90·0
	2. Sargondi .	21·6	67·8	13·1	11·5	0·23	2·0	88·0
	3. Mohanwal .	18·0	67·8	13·6	12·2	0·07	0·6	90·0
	4. Khurdpur .	23·8	67·4	13·7	12·2	0·15	1·2	89·0
	5. Rasulpur .	15·6	66·4	13·3	11·6	0·18	1·6	87·0
	*Jullundur A. S.	17·9	70·0	12·0	9·9	0·35	3·5	83·0
Average for the year	..	19·7	67·2	13·3	11·8	0·17	1·4	89·0
1932-33 . . .	*Jullundur A. S.	23·2	67·6	13·4	12·1	0·08	0·6	90·0
Average of two years	..	21·5	67·4	13·41	12·0	0·12	1·0	89·7

N.B.—Crops at Nos. 1, 2, 3, 4 and 5 in the year 1931-32 were attacked by white ants.
* Crop was badly lodged, the figures have not been taken into calculation.

TABLE XIV

Average composition of Co. 285 and Co. 290 during period of ripeness (Jullundur and Hoshiarpur districts)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
			Co. 285					
1929-30 . . .	Jullundur Farm	28·3	60·7	11·0	9·0	0·03	0·3	82·0
1930-31 . . .	Jullundur Farm	33·2	65·1	11·2	8·8	0·24	3·0	79·0
	Jullundur Farm (tenants area).	22·4	63·3	11·9	10·0	0·14	1·0	84·0
Average for the year	..	**27·8**	**64·2**	**11·6**	**9·4**	**0·19**	**2·0**	**81·0**
1931-32 . . .	Jabbowal .	31·6	61·7	11·6	9·6	0·41	4·3	83·0
	Sargondi .	36·5	61·7	11·8	9·9	0·37	3·8	84·0
	Chuheki . .	26·2	62·9	12·0	10·2	0·30	3·0	85·0
	Mohanwal .	33·7	62·0	11·8	10·0	0·19	1·9	85·0
	Rasulpur .	35·4	61·2	11·8	9·9	0·18	1·8	84·0
	Miani Afghanan	18·7	64·6	11·4	9·6	0·24	2·5	84·0
Average for the year	..	**30·4**	**62·4**	**11·7**	**9·9**	**0·28**	**2·8**	**84·4**

1932-33 . . .	Jullundur A. S.	28·4	64·2	11·4	8·9	0·35	4·0	78·0
	Mehli . .	29·2	62·6	12·2	10·7	0·22	2·4	87·0
	Chuheki . .	28·2	62·8	12·0	10·4	0·16	1·6	86·0
	Miani Afghanan (Ratoon)	20·2	62·8	11·5	9·6	0·19	2·0	83·0
	Khurdpur .	23·8	62·3	11·2	9·7	0·26	2·7	86·0
	Jullundur A. S.	31·9	63·1	11·4	9·4	0·26	2·8	82·0
Average for the year	..	26·7	62·7	11·7	10·0	0·22	2·2	85·4
Average of four years	..	28·3	62·5	11·5	9·5	0·18	1·9	83·0
			Co. 290					
1929-30 . . .	Jullundur A. S.	23·3	64·3	11·2	9·0	0·20	2·0	80·0
1930-31 . . .	Jullundur A. S.	27·4	70·5	10·7	8·4	0·58	7·0	78·0
1931-32 . . .	Jabbowal .	28·7	68·2	13·4	11·7	0·29	2·5	87·0
	Chuheki . .	16·1	68·4	13·1	11·2	0·40	3·6	86·0
	Mohanwal .	31·6	68·2	13·3	11·3	0·42	3·9	85·0
	Khurdpur .	35·9	70·7	13·3	11·2	0·47	4·0	84·0
Average for the year	..	28·1	68·9	13·3	11·4	0·40	3·5	85·3
Average of three years	..	26·3	67·9	11·7	9·6	0·39	4·1	81·7

TABLE XV

Amount of juice and sucrose, etc., per acre (in tons) and per ton of cane (Jullundur and Hoshiarpur districts)

Variety	Year	Weight per acre				Weight per ton of cane		
		Stripped canes	Juice	Total solids	Sucrose	Juice	Total solids	Sucrose
Katha	1927-28	23·0	14·20	2·76	2·37	0·619	0·120	0·103
	1928-29	12·1	6·88	1·25	1·04	0·569	0·103	0·086
	1929-30	14·4	7·65	1·50	1·27	0·531	0·104	0·088
	1930-31	12·9	7·28	1·35	1·11	0·564	0·105	0·086
	1931-32	16·5	9·49	1·81	1·48	0·575	0·110	0·090
	1932-33	15·7	8·97	1·71	1·38	0·571	0·109	0·088
	Average	**15·8**	**9·01**	**1·71**	**1·43**	**0·572**	**0·109**	**0·090**
Dhaulu	1927-28	24·6	15·40	2·58	2·22	0·626	0·105	0·090
Yuba	1927-28	27·3	18·34	3·34	2·96	0·671	0·122	0.108
Co. 223	1927-28	30·5	20·50	3·57	3·14	0·671	0·117	0·103
	1928-29	15·4	9·73	1·48	1·20	0·632	0·096	0·078
	1929-30	21·1	12·30	2·22	1·94	0·582	0·105	0·092
	1930-31	22·2	14·60	2·46	2·09	0·659	0·111	0·098
	1931-32	26·8	16·99	3·11	2·65	0·634	0·116	0·099
	1932-33	16·2	10·43	1·89	1·61	0·646	0·117	0·100
	Average	**22·0**	**14·04**	**2·47**	**2·08**	**0·637**	**0·112**	**0·095**
Co. 205	1927-28	26·3	16·28	3·02	2·50	0·619	0·115	0·095
	1928-29	16·9	10·36	1·71	1·35	0·613	0·101	0·080
	1929-30	21·8	12·51	2·46	2·07	0·574	0·113	0·095
	1930-31	22·3	13·71	2·50	2·01	0·615	0·112	0·090
	1931-32	23·9	14·30	2·67	2·13	0·599	0·112	0·089
	1932-33	21·5	12·99	2·49	2·00	0·604	0·116	0·093
	Average	**22·1**	**13·34**	**2·46**	**2·00**	**0·604**	**0·111**	**0·090**

Co. 213 . . .	1928-29 . .	15·1	9·56	1·59	1·27	0·633	0·105	0·084
	1929-30 . .	21·7	10·31	2·32	2·02	0·606	0·107	0·093
	1930-31 . .	19·5	13·10	2·40	2·09	0·671	0·123	0·107
	1931-32 . .	30·2	20·08	3·84	3·26	0·665	0·127	0·108
	1932-33 . .	22·4	14·94	2·78	2·40	0·667	0·124	0·107
	Average . .	**21·8**	**14·14**	**2·59**	**2·21**	**0·646**	**0·117**	**0·100**
Co. 285 . . .	1929-30 . .	28·3	17·18	3·12	2·56	0.607	0·110	0·090
	1930-31 . .	27·8	17·84	3·21	2·59	0·642	0·116	0·094
	1931-32 . .	30·4	18·94	3·55	3·00	0·624	0·117	0·099
	1932-33 . .	26·7	16·74	3·16	2·64	0·627	0·117	0·099
	Average . .	**28·3**	**17·68**	**3·25**	**2·70**	**0·625**	**0·115**	**0·96**
Co. 290 . . .	1929-30 . .	23·3	14·98	2·62	2·11	0·643	0·112	0·090
	1930-31 . .	27·4	19·30	2·94	2·30	0·705	0·107	0·084
	1931-32 . .	28·1	19·36	3·24	3·20	0·689	0·133	0·114
	Average . .	**26·3**	**17·88**	**2·90**	**2·54**	**0·679**	**0·118**	**0·096**
Co. 281 . . .	1931-32 . .	19·7	13·27	2·63	2·33	0·672	0·133	0·118
	1932-33 . .	23·2	15·68	3·11	2·81	0·676	0·134	0·121
	Average . .	**21·50**	**14·47**	**2·88**	**2·58**	**0·674**	**0·134**	**0·120**

TABLE XVI

Composition of juice from different varieties of cane—average of six years (Jullundur and Hoshiarpur districts)

Name of variety of cane	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
		Juice	Total solids	Sucrose	Glucose		
Katha	15·77	57·2	10·86	9·02	0·19	2·1	83·1
Dhaulu	24·60	62·6	10·49	9·02	0·06	0·6	86·0
Yuba	27·30	67·1	12·22	10·83	0·34	3·1	88·6
Co. 223	22·03	63·7	11·02	9·45	0·40	4·3	85·8
Co. 205	22·10	60·4	11·14	9·03	0·59	6·5	81·1
Co. 213	21·78	64·8	11·72	9·98	0·33	3·3	85·2
Co. 285	28·29	62·5	11·49	9·54	0·18	1·9	83·0
Co. 290	26·27	67·9	11·70	9 58	0·39	4·1	81·7
Co. 281	21·47	67·4	13·35	11·97	0·12	1·0	89·7
Local (Katha only)*	15·77	57·2	10·86	9·02	0·19	2·1	83·1
Coimbatore (average)	**23·65**	**64·5**	**11·74**	**9·93**	**0·33**	**3·3**	**84·6**
	Varietal tests at Jullundur Agricultural Station						
Co. 300 (1932-33)	27·2	67·2	12·9	11·3	0·26	2·4	87·0
Co. 301 („)	25·2	67·2	12·8	11·3	0·28	2·6	88·0
Co. 313 („)	23·4	67·1	13·4	11·7	0·25	2·3	88·0
Co. 312 („)	29·8	68·4	12·4	10·8	0·28	2·6	87·0

* Figures for Dhaulu and Yuba, being those from single samples only, have been neglected for calculating average.

TABLE XVII

Period of optimum ripeness for different varieties of cane (Jullundur and Hoshiarpur districts)

Year	Katha	Dhaulu	Yuba	Co. 223	Co. 205	Co. 213	Co. 285	Co. 290	Co. 281
1927-28 . .	10/1 to 5/2	8/1 to 13/3	8/2 to 28/3	18/2 to 14/3	7/3 to 5/4	..	..	..	..
1928-29 . .	1/12 to 26/2	..	..	1/12 to 15/3	14/12 to 3/4	15/12 to 23/3	..	..	..
1929-30 . .	23/11 to 3/3	..	..	14/2 to 9/4	16/2 to 8/4	8/2 to 7/4	28/2 to 1/4	7/1 to 22/4	..
1930-31 . .	27/11 to 2/3	..	..	20/12 to 15/3	1/2 to 10/4	4/2 to 18/4	2/2 to 9/4	28/1 to 10/4	20/1 to 1/4
1931-32 . .	30/11 to 7/4	..	..	28/1 to 5/3	15/1 to 21/4	8/2 to 22/4	27/12 to 15/4	18/12 to 15/4	16/12 to 6/4
1932-33 . .	4/10 to 30/3	..	..	8/1 to 6/4	..	20/1 to 15/4	20/12 to 22/4	4/1 to 4/4	7/12 to 25/4
Average period of optimum ripeness	21/11 to 8/3	8/1 to 13/3	8/2 to 28/3	15/1 to 21/3	10/2 to 10/4	22/1 to 13/4	20/1 to 12/4	7/1 to 13/4	24/12 to 11/4

Generally speaking the crushing season may start at the beginning of December and last till the middle of April, i.e., about 135 days.

TABLE XVIII

Average composition of Dhaulu and Yuba during period of ripeness (Gurdaspur Circle)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
			Dhaulu					
1927-28 . . .	Kaler Kalan .	9·7	51·9	9·03	7·84	0·36	4·6	86·8
	Sohal . .	10·4	52·0	9·67	8·43	0·22	2·6	87·2
	Tibbar . .	16·2	52·5	9·55	8·14	0·21	2·6	85·2
Average for the year	..	**12·1**	**52·1**	**9·42**	**8·14**	**0·26**	**3·2**	**86·4**
1928-29 . . .	Chhina . .	4·8	51·2	9·22	8·09	0·21	2·6	87·7
	Sheikhpur ..	12·1	55·9	9·75	8·16	0·17	2·1	83·7
Average for the year	..	**8·5**	**53·6**	**9·49**	**8·13**	**0·19**	**2·3**	**85·7**
1929-30	Chhina . .	7·4	52·5	8·70	7·60	0·20	2·6	87·3
	Bhopalwala .	15·8	54·5	9·80	8·20	0·30	3·7	83·7
Average for the year	..	**11·6**	**53·5**	**9·30**	**7·90**	**0·25**	**3·2**	**84·9**
1930-31 . . .	Chhina . .	13·9	47·8	8·96	7·60	0·27	3·6	84·8
	Bhopalwala .	16·9	56·6	10·91	9·08	0·41	4·5	3·2
Average for the year	..	**15·4**	**52·2**	**9·94**	**8·34**	**0·34**	**4·1**	**83·9**

1931-32 . . .	Bhopalwala .	10·8	57·7	9·70	7·80	0·43	5·5	80·4
	Begowal . .	12·8	57·6	10·80	9·00	0·32	3·6	83·3
Average for the year	..	11·8	57·6	10·20	8·40	0·38	4·6	82.3
Average of five years	..	11·9	53·8	9·67	8·18	0.28	3·4	84.6
Average yield for the last four years	..	11·9	..	..	..	..	..	..
				Yuba				
1927-28 . . .	Sohal . .	14·1	62·3	11·03	9·21	0·77	8·4	83.5
1928-29 . . .	Daulatpur .	10·3	64·7	11·78	10·10	0·52	5·2	85.7
Average of two years	..	12.2	63·5	11·40	9·65	0·64	6·6	84.6

TABLE XIX

Average composition of Co. 205 during period of ripeness (Gurdaspur Circle)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
1927-28 . . .	Sangatpur .	21·0	57·2	10·12	7·49	0·46	6·1	74·0
	Nawapind .	16·2	59·0	10·27	8·44	0·77	9·1	82·2
	Dhakki . .	15·0	54·9	9·70	8·23	0·38	4·6	84·8
	Gurdaspur .	18·4	55·5	10·10	8·36	0·51	6·1	82·8
Average for the year	..	**17·7**	**56·7**	**10·05**	**8·13**	**0·53**	**6·5**	**80.9**
1928-29 . . .	Haveli Chobdaran.	13·0	60·0	10·81	9·00	0·84	9·3	83·3
	Pakkhowal .	12·3	60·1	9·92	7·75	0·96	12·4	78·1
	Shahidpur .	10·3	61·1	10·51	8·55	0·79	9·2	81·4
Average for the year	..	**11·9**	**60·4**	**10·41**	**8·43**	**0.86**	**10·2**	**81·0**
1929-30 . . .	Ujela . .	17·5	58·6	9·60	8·20	0·80	9·8	85·4
	Haveli Chobdaran.	17·0	58·7	10·80	8·90	0·90	10·1	82·4
	Sangatpur .	17·8	61·3	11·30	9·60	0·70	7·3	84·9

	Chandowal .	12·5	57·1	10·10	8·40	0·60	7·1	83·2
	Bhopalwala .	20·0	56·7	9·70	7·30	0·80	11·0	75·2
Average for the year	..	17·0	58·5	10·30	8·48	0·76	9·0	82.3
1930-31 .	Haveli Chobdaran.	16·6	59·5	11·17	9·20	0·52	5·7	82·3
	Sialkot . .	31·4	58·7	10·80	9·05	0·25	2·8	83·8
	Bhopalwala .	20·1	56·2	10·90	9·03	0·57	6·3	82·8
Average for the year.	..	22·7	58·1	10·96	9·09	0·45	5·0	82·9
1931-32 . .	Bhopalwala .	20·0	63·3	12·30	10·20	0·65	6·3	83·6
	Gujranwala A. S.	14·8	59·5	10·30	8·40	0·39	4·6	81·6
Average for the year.	..	17·4	61·4	11·30	9·30	0·52	5·6	82·2
Average of the last five years.	..	17·34	59·0	10·60	8·69	0·62	7·1	81.9
Average yield for the last four years.	..	17·25						

TABLE XX

Average composition of Co. 213 during period of ripeness (Gurdaspur Circle)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
1927-28 . . .	Gurdaspur .	18·2	63·1	11·35	9·69	0·44	4·8	85·4
1931-32 . . .	Nawanpind .	15·4	63·9	11·8	10·2	0·42	4·1	86·4
	Chhina . .	20·2	65·4	12·1	10·3	0·64	6·2	85·1
Average for two years	..	17·8	64·6	11·95	10·25	0·53	5·2	85·8
1932-33 . . .	Gurdaspur A. S.	17·5	67·0	11·5	10·0	0·45	4·5	87·8
	Nawanpind .	23·1	67·2	12·7	10·8	0·48	4·4	85·0
	Chhina . .	13·7	66·6	11·5	10·0	1·02	10·2	86·9
	Gujranwala A. S.	14·1	66·3	12·0	10·7	0·20	1·9	89·2
	* Rajah Sansi (Red Rot).	18·7	70·8	7·7	5·4	1·39	25·7	70·1
Average for the year.	..	17·1	66·8	11·9	10·4	0·54	5·3	87·4
Average of three years	..	17·7	64·9	11·73	10·11	0·50	4·9	86·2
Average yield for the last two years	..	17·5	..	..	..	..	..	..

* Sample abnormal, figures not taken into calculation when computing averages.

TABLE XXI

Average composition of Co. 223 during period of ripeness (Gurdaspur Circle)

Year	Locality	Weight of stripped cane in tons per acre	PERCENTAGE ON CANE				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
1927-28 . . .	Dhariwal. .	18·2	60·1	10·33	8·65	0·60	6·9	83·7
	Bham . .	26·0	62·3	10·97	9·41	0·37	3·9	85·8
	Dariwala .	21·0	61·4	10·44	8·72	0·61	7·0	83·5
	Qadian . .	23·0	60·9	10·38	8·71	0·58	6·7	83·9
	Tibbar . .	17·4	62·2	10·70	9·27	0·56	6·0	86·6
	Dhakki . .	15·5	59·5	10·05	8·89	0·36	4·1	88·3
	Gurdaspur .	20·0	62·9	10·11	9·04	0·58	6·4	89·4
Average for the year.	..	**20·2**	**61·3**	**10·43**	**8·95**	**0·52**	**5·8**	**85·8**
1928-29 . . .	Sheikhupur .	18·2	63·1	10·98	9·40	0·32	3·4	85·6
	Haveli Chobdaran.	21·0	64·9	10·77	9·35	0·45	4·8	86·8
	Daulatpur .	20·3	62·7	10·35	8·65	0·50	5·8	83·6
	Chhina . .	14·7	62·0	9·86	8·18	0·62	7·6	83·0
	Gurdaspur .	22·7	65·6	10·76	9·32	0·59	6·3	86·6

TABLE XXI—*contd.*

Average composition of Co. 223 during period of ripeness (Gurdaspur Circle)—contd.

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
Average for the year.	..	**19·4**	**63·7**	**10·54**	**8·98**	**0·50**	**5·6**	**85·2**
1929-30 . . .	Gurdaspur .	22·1	63·4	8·50	7·20	0·80	11·1	84.7
	Chhina . .	10·5	60·4	9·80	8·40	0·80	9·5	85·7
	Nawanpind .	15·3	61·5	8·60	6·00	0·70	11·6	69·8
	Haveli Chobdaran.	17·7	63·1	10·40	8·80	1·0	11·4	84·6
	Adamke . .	20·4	61·2	9·50	8·20	0·50	6·1	86·3
Average for the year.	..	**17·2**	**61·9**	**9·36**	**7·72**	**0·76**	**9·9**	**82·5**
1930-31 . . .	Gurdaspur .	18·6	67·5	11·10	9·70	0·66	6·8	87·4
	Nawanpind .	16·9	63·7	11·25	9·47	0·85	9·0	84·2
	Chhina . .	19·1	64·8	10·63	8·96	0·87	9·7	84·3
	Haveli Chobdaran.	16·8	65·1	11·46	9·93	0·51	5·2	86·7
	Sialkot . .	15·4	64·4	11·36	9·90	0·37	3·7	87·2
	Chandowal .	16·2	65·1	10·62	8·84	0·72	8·2	83·2
	Bhopalwala .	20·3	63·9	10·69	9·19	0·59	6·4	86·0

Average for the year.	..	**17·6**	**64·9**	**11·02**	**9·43**	**0·65**	**6·9**	**85·7**
1931-32 . . .	Gurdaspur A. S.	16·8	59·9	11·00	9·70	0·27	2·8	88·1
	Gurdaspur A. S.	12·3	60·0	10·40	9·20	0·40	4·4	88·5
	Nawanpind .	16·7	61·0	11·00	9·40	0·58	6·2	85·4
	Chhina . .	18·5	63·5	11·80	9·90	0·84	8·5	83·9
	Bhopalwala .	14·4	64·5	11·30	9·51	0·55	5·8	84·0
	Begowal . .	12·2	54·7	9·60	7·82	0·42	5·5	81·2
	Gujranwala A. S.	13·6	65·3	10·90	9·43	0·31	3·3	86·2
	Raja Sansi .	20·3	65·5	10·90	9·24	0·53	5·8	84·4
Average for the year.	..	**15·6**	**61·8**	**10·85**	**9·26**	**0·49**	**5·3**	**85·4**
1932-33 . . .	Gurdaspur A. S.	19·2	65·1	10·70	9·44	0·63	6·7	87·8
	Nawanpind .	22·0	67·1	11·20	9·01	1·03	11·4	80·3
	Chhina . .	14·5	63·9	10·90	9·10	0·80	8·8	83·5
	Chandowal .	14·4	65·7	11·50	9·63	1·00	10·4	83·5
	Sialkot . .	18·5	64·3	11·10	9·21	0·38	4·3	88·3
	Gujranwala A. S.	13·5	64·0	11·00	10·08	0·17	1·7	91·0
	Rajah Sansi .	20·0	63·6	11·70	10·01	0·59	5·9	84·1
	Doburji . .	18·9	62·9	11·60	9·90	0·41	4·1	83·4
Average for the year.	..	**17·6**	**64·6**	**11·20**	**9·55**	**0·62**	**6·7**	**85·1**
Average of six years	..	**17·9**	**63·03**	**10·57**	**8·99**	**0·59**	**6·57**	**85·0**
Average yield for the last five years.	..	**17·5**	..	..	..	..	..	..

TABLE XXII

Average composition of Co. 285, Co. 214 and Co. 300 during period of ripeness (Gurdaspur Circle)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
				Co. 285				
1929-30 . . .	Gurdaspur .	22·4	63·6	10·10	8·70	0·50	5·8	86·1
	Chhina . .	12·2	60·7	10·90	9·30	0·40	4·3	85·3
	Nawanpind .	13·3	62·1	10·30	8·70	0·60	6·9	84·5
	Haveli Chobdaran	18·8	62·7	12·50	10·90	0·40	3·7	87·2
	Chandowal .	15·2	59·6	11·10	9·80	0·30	3·1	88·3
Average for the year.	..	**16·4**	**61·8**	**11·00**	**9·48**	**0·44**	**4·8**	**86·2**
1930-31 . . .	Gurdaspur .	24·0	64·1	11·60	10·00	0·50	5·0	86·2
	Nawanpind .	18·4	69·3	11·01	9·37	0·41	4·4	85·1
	Chhina . .	19·6	60·6	10·78	8·98	0·70	7·8	83·3
	Haveli Chobdaran	18·8	62·4	11·77	10·31	0·30	2·9	87·6
	Sialkot . .	19·6	62·2	11·25	9·64	0·35	3·6	85·7
	Chandowal .	23·5	62·1	11·16	9·73	0·49	5·0	87·2

Average for the year.	..	20·7	63·5	11·26	9·67	0·46	4·8	85·9
1931-32 . . .	Gujranwala A.S.	14·8	60·9	11·30	9·60	0·20	2·0	85·0
	Gurdaspur A. S.	22·0	59·3	11·20	9·60	0·27	2·8	85·7
	Nawanpind .	22·8	61·9	11·40	9·50	0·48	5·1	83·4
	Rajah Sansi .	26·5	61·9	11·80	9·80	0·31	3·2	83·0
	Chhina . .	21·5	60·1	11·40	9·50	0·58	6·1	83·3
Average for the year.	..	21·5	60·8	11·40	9·60	0·37	3·9	84·2
1932-33 . . .	Gurdaspur A. S.	24·2	61·7	10·60	9·20	0·50	5·4	86·8
	Nawanpind .	25·9	61·3	12·40	10·70	0·28	2·6	86·3
	Chhina . .	17·6	63·3	10·90	9·30	0·63	6·9	85·3
	Chandowal .	20·0	62·2	11·70	9·90	0·42	4·3	84·6
	Sialkot A. S. .	23·5	60·9	11·60	10·20	0·23	2·2	88·0
	Gujranwala A.S.	20·0	60·2	11·40	10·20	0·16	1·6	89·5
	Rajah Sansi .	24·8	62·6	10·60	9·00	0·41	4·5	84·9
	Doburji . .	22·0	62·0	11·40	9·70	0·42	4·3	85·1
Average for the year.	..	22·25	61·8	11·30	9·80	0·38	3·9	86·7
Average of four years	..	20·21	61·6	11·24	9·64	0·41	4·3	85·8
1927-28 . . .	Gurdaspur .	10·0	56·4	*Co. 214* 11·11	9·73	0·18	1·9	87·6
1932-33 . . .	Nawanpind .	22·7	64·9	*Co. 300* 12·0	10·5	0·70	6·8	87·5
	Rajah Sansi * .	19·7	69·2	7·9	5·9	0·74	12·5	85·5

* The figures of analyses have not been taken into consideration as the sample was abnormal.

TABLE XXIII

Average composition of Co. 290 during period of ripeness (Gurdaspur Circle)

Year	Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
			Juice	Total solids	Sucrose	Glucose		
1929-30 . . .	Gurdaspur .	19·1	67·1	9·90	8·50	0·50	5·9	85·9
	Chandowal .	14·2	67·5	12·30	11·20	0·30	2·7	91·0
Average for the year.	..	**16·7**	**67·3**	**11·10**	**9·85**	**0·40**	**4·1**	**88·7**
1930-31 . . .	Gurdaspur .	20·8	70·1	12·70	11·23	0·57	5·1	88·4
	Sialkot . .	11·8	67·0	12·80	11·50	0·38	3·3	89·8
	Chandowal .	19·4	66·0	12·26	10·95	0·45	4·1	89·3
Average for the year.		**17·3**	**67·7**	**12·59**	**11·23**	**0·47**	**4·2**	**89·2**
1931-32 . . .	Gurdaspur A. S.	16·2	65·5	12·00	10·20	0·63	6·2	85·2
	Nawanpind .	20·8	68·6	12·80	11·10	0·24	2·2	86·7
	Rajah Sansi .	23·7	66·2	12·60	10·80	0·23	2·1	85·7
Average for the year.	..	**20·2**	**66·8**	**12·50**	**10·70**	**0·37**	**3·5**	**85·6**
1932-33 . . .	Gurdaspur A. S.	19·0	69·3	12·30	10·90	0·43	3·9	88·6
	Nawanpind .	22·0	69·2	12·74	11·00	0·61	5·5	86·6
	Chandowal .	18·8	67·2	12·81	11·23	0·41	3·7	87·5
	Sialkot . .	24·0	68·4	12·50	11·24	0·24	2·2	89·6
	Gujranwala .	21·2	68·4	12·01	11·02	0·21	1·9	89·0
	Rajah Sansi .	22·7	69·0	11·73	10·01	0·40	4·0	86·3
Average for the year.	..	**21·3**	**68·6**	**12·35**	**10·90**	**0·38**	**3·5**	**88·2**
Average of four years	..	**18·9**	**67·6**	**12·14**	**10·67**	**0·41**	**3·8**	**87·9**

TABLE XXIV

Amount of juice, sucrose, etc., per acre (in tons) and per ton of cane (Gurdaspur Circle)

Variety	Year	Weight per acre				Weight per ton of cane		
		Stripped cane	Juice	Total solids	Sucrose	Juice	Total solids	Sucrose
Dhaulu	1927-28	12.10	6.30	1.14	0.98	0.521	0.094	0.081
	1928-29	8.50	4.56	0.81	0.69	0.536	0.095	0.081
	1929-30	11.60	6.22	1.08	0.92	0.535	0.093	0.079
	1930-31	15.40	8.04	1.45	1.28	0.522	0.094	0.083
	1931-32	11.80	6.80	1.25	1.04	0.576	0.106	0.084
	Average	**11.88**	**6.40**	**1.15**	**0.98**	**0.538**	**0.096**	**0.082**
Yuba	1927-28	14.10	8.79	1.57	1.30	0.623	0.110	0.092
	1928-29	10.30	6.66	1.22	1.04	0.647	0.118	0.101
	Average	**12.20**	**7.75**	**1.39**	**1.17**	**0.635**	**0.114**	**0.096**
Co. 223	1927-28	20.16	12.36	2.10	1.81	0.613	0.104	0.090
	1928-29	19.40	12.36	2.04	1.75	0.637	0.105	0.090
	1929-30	17.20	10.65	1.62	1.32	0.619	0.094	0.077
	1930-31	17.60	11.42	1.94	1.65	0.649	0.110	0.094

TABLE XXIV—*contd.*

Amount of juice sucrose, etc., per acre (in tons) and per ton of cane (Gurdaspur Circle)—contd.

Variety	Year	Weight per acre				Weight per ton of cane		
		Stripped cane	Juice	Total solids	Sucrose	Juice	Total solids	Sucrose
	1931-32 . .	15.60	9.64	1.70	1.47	0.618	0.109	0.093
	1932-33 . .	17.60	11.32	1.97	1.67	0.646	0.112	0.095
	Average . .	**17.93**	**11.30**	**1.89**	**1.62**	**0.631**	**0.106**	**0.090**
Co. 205 . . .	1927-28 . .	17.65	10.00	1.78	1.43	0.567	0.101	0.081
	1928-29 . .	11.90	7.19	1.24	1.00	0.604	0.104	0.084
	1929-30 . .	17.00	9.99	1.75	1.45	0.585	0.103	0.085
	1930-31 . .	22.70	13.20	2.50	2.07	0.581	0.110	0.091
	1931-32 . .	17.40	10.72	1.97	1.60	0.614	0.113	0.093
	Average . .	**17.33**	**10.22**	**1.85**	**1.50**	**0.590**	**0.107**	**0.087**
Co. 213 . . .	1927-28 . .	18.20	11.50	2.08	1.77	0.631	0.114	0.097
	1931-32 . .	17.80	11.50	2.14	1.83	0.646	0.120	0.103
	1932-33 . .	17.10	11.42	2.04	1.78	0.668	0.119	0.104
	Average . .	**17.70**	**11.47**	**2.09**	**1.80**	**0.648**	**0.118**	**0.101**

Co. 214 . . .	1927-28 . .	10.00	5.64	1.11	0.97	0.564	0.111	0.097
Co. 285 . . .	1929-30 . .	16.40	10.01	1.80	1.53	0.618	0.110	0.085
	1930-31 . .	20.70	12.80	2.34	2.01	0.618	0.113	0.096
	1931-32 . .	21.50	13.07	2.45	2.06	0.608	0.114	0.097
	1932-33 . .	22.25	13.75	2.51	2.18	0.618	0.113	0.098
	Average . .	**20.21**	**12.46**	**2.28**	**1.97**	**0.616**	**0.113**	**0.097**
Co. 290 . . .	1929-30 . .	16.70	11.20	1.85	1.65	0.673	0.111	0.099
	1930-31 . .	17.30	11.70	2.18	1.93	0.677	0.126	0.112
	1931-32 . .	20.20	13.49	2.53	2.16	0.668	0.125	0.107
	1932-33 . .	21.30	14.61	2.64	2.32	0.686	0.124	0.109
	Average . .	**18.88**	**12.75**	**2.30**	**2.04**	**0.676**	**0.122**	**0.107**
Co. 300 . . .	1932-33 . .	22.70	14.73	2.72	2.38	0.649	0.120	0.105

TABLE XXV

Composition of juice from different varieties of cane—average of six years (Gurdaspur Circle)

Variety of cane	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity co-efficient
		Juice	Total solids	Sucrose	Glucose		
Dhaulu	11.88	53.8	9.67	8.18	0.28	3.4	84.6
Yuba	12.20	63.5	11.40	9.65	0.64	6.6	84.6
Co. 223	17.93	63.0	10.57	8.99	0.59	6.6	85.0
Co. 205	17.34	59.0	10.60	8.69	0.62	7.1	81.9
Co. 213	17.70	64.9	11.73	10.11	0.50	4.9	86.2
Co. 285	20.21	61.6	11.24	9.64	0.41	4.3	85.8
Co. 290	18.98	67.6	12.14	10.67	0.41	3.8	87.9
Co. 214	10.00	56.4	11.11	9.73	0.18	1.9	87.6
Co. 300	22.70	64.9	12.00	10.50	0.70	6.8	87.5
Local (Average)	12.04	58.6	10.53	8.91	0.46	5·2	84·6
Coimbatore (Average). . . .	17.82	62.5	11.34	9.76	0.49	5.0	86.1

TABLE XXVI

Period of optimum ripeness for different varieties of cane (Gurdaspur Circle)

Year	Dhaulu	Yuba	Co. 223	Co. 205	Co. 213	Co. 214	Co. 285	Co. 290	Co. 300
1927-28 . .	3/1 to 27/2	15/12 to 30/1	1/2 to 7/4	27/1 to 1/4	15/1 to 15/3	10/1 to 30/2	..	..	..
1928-29 . .	6/12 to 9/2	4/1 to 28/2	1/1 to 16/3	26/12 to 30/3	..	..	..	..	..
1929-30 . .	28/12 to 19/1		4/3 to 30/4	20/1 to 13/4	..	..	10/1 to 15/4	15/1 to 18/4	..
1930-31 . .	21/11 to 24/2	..	15/12 to 6/4	17/3 to 1/4	..	..	5/12 to 9/4	6/12 to 5/4	..
1931-32 . .	27/11 to 20/1	..	1/12 to 20/3	1/2 to 30/3	5/1 to 3/4	..	10/12 to 20/3	20/12 to 20/2	15/12 to 20/2
1932-33 . .	..	..	9/12 to 21/4	..	19/1 to 15/4	..	15/12 to 29/4	15/12 to 21/4	..
Average period of optimum ripeness	**10/12 to 10/2**	**25/12 to 15/2**	**3/1 to 30/3**	**31/1 to 2/4**	**8/1 to 1/4**	**10/1 to 30/2**	**30/12 to 10/4**	**22/12 to 28/3**	**15/12 to 20/2**

Generally speaking the crushing season may start in the second week of December and last till the second week of April, *i.e.*, about 120 days.

TABLE XXVII

Showing the effect of frost on Dhaulu (Chhina, District Gurdaspur)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks		
		Juice	Sucrose	Glucose	Total sugars	Total solids					
									Frost on	Rain	On
5th Dec. 1928 .	..	50·9	7·6	0·26	7·9	9·2	3·3	82·7	24-25th Dec. 1928	0.85in.	1st Dec. 1928
									29th Dec. 1928 .	0.74in.	2nd Dec. 1928
19th Dec. 1928 .	..	49·0	7·7	0·15	7·9	8·9	1·9	86·8	6th Jan. 1929 .	0.05in.	29th Dec. 1928
									22nd Jan. 1929 .	0.09in.	26th Jan. 1929
2nd Jan. 1929 .	..	49·6	7·6	0·25	7·9	7·9	3·2	85·5	31st Jan. 1929 (H.F.*)	0.03in.	30th Jan. 1929
									1st-2nd Feb. 1929	0.07in.	9th Feb. 1929
16th Jan. 1929 .	..	54·0	9·1	0·11	9·2	9·2	1·1	89·8	(H. F.*)	0.06in.	13th Feb. 1929
									4.6th Feb. 1929 .		
30th Jan. 1929 .	..	49·4	8·8	0·14	8·9	8·9	1·6	93·2	13th Feb. 1929 .		
									15th Feb. 1929.		
3rd Feb. 1929 .	..	52·9	5·6	0·21	5·8	9·6	2·4	89·5	*H. F.=Heavy frost.		
13th Feb. 1929 .	..	53·0	7·5	0·42	7·9	8·7	5·7	84·7			
3rd Mar. 1929 .	..	50·5	5·8	0·40	6·2	8·1	7·0	71·2			
15th Mar. 1929 .	..	45·4	4·3	0·82	5·1	6·1	18·9	70·9			
27th Mar. 1929 .	..	45·4	3·7	1·27	5·0	5·4	34·5	68·6			
10th April 1929.	..	38·4	2·0	1·73	3·7	4·14	84·9	46·1			
Average for the period of maximum purity	4.8	51·2	8·09	0·21	8·3	9·22	2·53	87·7			

TABLE XXVIII

Showing the effect of frost on Katha (Khudda, District Hoshiarpur)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks		
		Juice	Sucrose	Glucose	Total sugars	Total solids					
									Frost on	Rain	On
23rd Nov. 1928 .	..	57·0	8·4	traces	8·4	10·2	0	82	27th-29th Dec. 1928	0.12in.	28th Nov. 1928
									6-10th Jan. 1929 .	0.53in.	29th Nov. 1928
5th Dec. 1928 .	..	58·5	8·8	,,	8·8	10·3	0	86	21st-23rd Jan. 1929	1.10in.	30th Nov. 1928
									31st Jan. 1929 .	0.71in.	1st Dec. 1928
19th Dec. 1928 .	..	58·6	9·0	,,	9·0	10·8	0	84	1st-2nd Feb. 1929	0.16in.	2nd Dec. 1928
									(H. F.)*	0.21in.	28th Dec. 1928
2nd Jan. 1929 .	..	61·5	9·2	,,	9·2	11·2	0	81	12th Feb. 1929 .	0.56in.	30th Dec. 1928
										0.07in.	24th Jan. 1929
16th Jan. 1929 .	..	60·3	9·2	,,	9·2	10·8	0	85		0.25in.	25th Jan. 1929
										0.10in.	26th Jan. 1929
30th Jan. 1929 .	..	60·8	8·9	,,	8·9	10·7	0	83		0.62in.	29th Jan. 1929
										0.07in.	8th Feb. 1929
1st Feb. 1929 .	..	60·0	8·2	0·23	8·4	10·2	3	80		0.03in.	10th Feb. 1929
14th Feb. 1929 .	..	61·1	8·4	0·43	8·8	10·4	5	80			
28th Feb. 1929 .	..	52·9	6·4	0·42	6·8	8·9	7	72			
Average for the period of maximum purity	15·0	59·5	8·8	0·30	9·11	10·59	0·4	83	*H. F.=Heavy frost.		

TABLE XXIX.

Showing the effect of frost on Katha (Jabbowal, District Hoshiarpur)

Date of sampling	Weight of stripped cane per acre in tons	PERCENTAGE ON CANE (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks
		Juice	Sucrose	Glucose	Total sugars	Total solids			
24th Nov. 1930	..	53·5	7·9	0·14	8·0	10·3	1·8	77	Cane was attacked by top borer and smut.
8th Dec. 1930	..	54·7	8·6	0·11	8·7	10 5	1·3	82	
19th Dec. 1930	..	56·6	8·2	0·54	8·7	10·9	6·6	75	
4th Jan. 1931	.	59·4	9·0	0·54	9·5	11·0	6·0	82	
18th Jan. 1931	..	54·4	6·9	0·97	7·9	10·0	14·0	69	
1st Feb. 1931	..	58·6	8·9	0·52	9·4	11·3	5·8	79	
21st Feb. 1931	..	57·9	8·7	0·30	9·0	10·8	3·4	80	
6th Mar. 1931	..	56·1	9·4	0·44	9·8	11·3	4·8	83	
17th Mar. 1931	..	56·8	9·2	0·38	9·6	11·4	4·1	81	
31st Mar. 1931	..	53·7	9·2	0·53	9·7	11·3	5·7	81	
19th April 1931	.	55·6	8·0	0·43	9·4	11·7	17·9	68	
24th Nov. 1930 to 8th Dec. 1930. (Average during maximum purity period)	12·6	54·1	8·3	0·13	8·4	10·4	1·6	80	

Frost on	Rain	On
8th Dec. 1930 (H. F.*)	1.40in.	30th Dec. 1930
12th Dec. 1930 .	0.07in.	10th Jan. 1931
19th-21st Dec. 1930	0.19in.	24th Jan. 1931
22nd-27th Dec. 1930	0.32in.	25th Jan. 1931
1st-2nd Jan. 1931	0.11in.	1st Feb. 1931
16-17th Jan. 1931	0.09in.	3rd Feb. 1931
29th Jan. 1931 .	0.34in.	8th Feb. 1931
31st Jan. 1931 (H.F.*)	0.07in.	9th Feb. 1931
	0.35in.	18th Feb. 1931
	0.29in.	24th Feb. 1931
	0.31in.	25th Feb. 1931
	0.19in.	3rd Mar. 1931
	0.90in.	4th Mar. 1931

*H. F.=Heavy frost.

TABLE XXX

Showing the effect of moderate cold, following heavy frost on Co. 223 (Gurdaspur Agricultural Station)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks		
		Juice	Sucrose	Glucose	Total sugars	Total solids					
5th Nov. 1930 .	..	66·7	7·8	1·2	9·0	9·9	15·4	78·8	Frost on	Rain	On
20th Nov. 1930 .	..	66·4	7·5	1·2	8·7	9·5	16·0	78·9	7-12th Dec. 1930 .	0·05in.	29th Dec. 1930
2nd Dec. 1930 .	..	64·3	7·3	1·1	8·4	9·3	15·0	78·5	20th-21st Dec. 1930	0·12in.	21st Jan. 1931
18th Dec. 1930 .	..	64·3	8·6	0·8	9·4	10·4	9·3	82·7	22nd-27th Dec. 1930	0·40in.	24th Jan. 1931
30th Dec. 1930 .	..	62·4	8·1	0·9	9·0	9·7	11·1	83·5	1st Jan. 1931 .	0·20in.	25th Jan. 1931
14th Jan. 1931 .	..	66·0	9·6	0·7	10·3	11·1	7·5	86·5	16th Jan. 1931 .	0·41in.	3rd Feb. 1931
28th Jan. 1931 .	..	68·9	9·8	0·6	10·4	11·4	6·1	83·9	27th Jan. 1931 .	0·37in.	8th Feb. 1931
11th Feb. 1931 .	..	67·2	9·6	0·7	10·3	11·3	7·3	84·9	29-30th Jan. 1931	0·04in.	9th Feb. 1931
28th Feb. 1931 .	..	66·8	9·1	0·7	9·8	10·6	7·7	85·8	4-5th Feb. 1931 .	0·74in.	18th Feb. 1931
12th Mar. 1931	..	66·3	9·7	0·4	10·1	11·2	4·1	86·6	20th-21st Feb. 1931	0·05in.	19th Feb. 1931
25th Mar. 1931 .	..	67·3	10·5	0·4	10·9	11·6	5·8	90·5		0·78in.	24th Feb. 1931
8th Apl. 1931 .	..	67·4	10·8	0·4	11·2	11·9	3·7	90·7		0·22in.	25th Feb. 1931
22nd Apl. 1931 .	..	63·8	10·7	0·3	11·0	11·6	2·8	92·2			
14th Jan. 1931 to 22nd Apl. 1931 (Average during the maximum purity period.)	29·07	66·71	9·97	0·52	10·49	11·33	5·26	88·0			

TABLE XXXI

Showing the effect of moderate cold following heavy frost on Co. 223 (Gurdaspur Agricultural Station)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks
		Juice	Sucrose	Glucose	Total sugars	Total solids			
5th Nov. 1930 .	..	67·6	7·7	1·3	9·0	10·1	16·9	76·2	Manured with ammonium sulphate at 1½ mds. per acre
20th Nov. 1930 .	..	65·6	7·7	1·0	8·7	9·6	13·0	80·2	Frost and rainfall as in Table XXX
2nd Dec. 1930 .	..	64·4	7·0	1·1	8·1	8·9	15·7	78·6	
18th Dec. 1930 .	..	64·9	7·3	1·5	8·8	9·5	20·5	76·8	
30th Dec. 1930 .	..	64·4	7·4	1·1	8·5	9·7	14·9	76·2	
14th Jan. 1931 .	..	66·0	7·4	1·5	8·9	9·8	28·2	75·5	
28th Jan. 1931 .	..	70·4	8·5	1·2	9·7	10·7	14·1	79·4	
11th Feb. 1931 .	..	70·8	8·7	1·2	9·9	11·1	13·8	78·4	
28th Feb. 1931 .	..	67·2	9·5	0·8	10·3	11·2	8·4	84·8	
12th Mar. 1931 .	..	68·5	9·9	0·5	10·4	11·6	5·1	85·3	
25th Mar. 1931	..	67·6	9·8	0·6	10·4	11·3	6·1	86·7	
8th Apl. 1931 .	..	66·8	10·6	0·4	11·0	12·0	3·7	88·3	
22nd Apl. 1931 .	..	64·5	11·2	0·3	11·5	11·9	2·7	94·1	
28th Feb. 1931 to 22nd Apl. 1931 (Average during the maximum purity period)	31·78	66·92	10·20	0·52	10·72	11·6	3·1	87·9	

TABLE XXXII

Showing the delayed ripening of Katha due to frost followed by moderate cold (Chandowal, Sialkot)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks		
		Juice	Sucrose	Glucose	Total sugars	Total solids					
6th Dec. 1932 .	..	57·6	9·2	0·52	9·7	11·2	5·7	82·1	Frost on	Rain	On
20th Dec. 1932 .	..	59·0	7·5	0·85	8·4	10·2	11·3	73·5	4th Dec. 1932 .	2·0 in.	24th Dec. 1932
5th Jan. 1933 .	..	53·5	8·7	0·71	9·4	10·5	8·2	82·8	19-11th Dec. 1932 .	0·1 in.	27th Dec. 1932
17th Jan. 1933 .	..	59·6	6·4	1·24	7·6	9·2	19·4	69·8	16-17th Dec. 1932	0·1 in.	30th Dec. 1932
31st Jan. 1933 .	..	62·2	8·2	1·38	9·6	10·7	16·8	76·6	3rd Jan. 1933 .	0·4 in.	18th Jan. 1933
14th Feb. 1933 .	..	56·8	8·7	0·68	9·4	10·1	7·8	86·1	6th Jan. 1933 .	0·1 in.	19th Jan. 1933
5th Mar. 1933 .	..	60·1	6·4	0·86	7·3	10·3	13·4	62·2	8th Jan. 1933 .	0·1 in.	11th Feb. 1933
21st Mar. 1933 .	..	54·1	7·3	0·51	7·8	9·7	7·0	75·2	11-14th Jan. 1933 .	0·2 in.	13th Feb. 1933
4th Apl. 1933 .	..	55·9	10·0	0·47	10·5	11·3	4·7	88·5	15-17th Jan. 1933 .	0·2 in.	25th Feb. 1933
4th Apl. 1933	12·7	55·9	10·0	0·47	10·5	11·3	4·7	88·5	(H. F.)*	0·1 in.	26th Feb. 1933
Average during the period of ripeness									22nd Jan. 1933 .	0·8 in.	8th Mar. 1933
									25th-31st Jan. 1933	0·1 in.	20th Mar. 1933
										0·1 in.	24th Mar. 1933
										1·0 in.	25th Mar. 1933
									* H. F.-Heavy frost.		

TABLE XXXIII

Showing the effect of frost on Pyrilla *affected Lalri canes (Lehrara, Rohtak)*

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks		
		Juice	Sucrose	Glucose	Total sugars	Total solids					
									Frost on	Rain	On
7th Nov. 1930 .	..	58·5	6·99	0·27	7·26	9·77	3·83	71·5	23rd-25th Dec. 1930	0·13 in.	11th Feb. 1931
									1st-4th Jan. 1931 .	0·20 in.	3rd Mar. 1931
										0·50 in.	4th Mar. 1931
27th Nov. 1930 .	..	56·9	7·09	0·18	7·27	9·10	2·54	78·0			
4th Dec. 1930 .	..	55·7	6·62	0·31	6·93	8·90	4·68	74·4			
17th Dec. 1930 .	.	58·3	7·53	0·32	7·85	9·32	4·25	80·7			
4th Jan. 1931 .	.	55·7	6·88	0·75	7·63	9·12	10·90	75·4			
21st Jan. 1931 .	..	55·4	5·62	0·78	6·40	9·23	15·60	61·0			
7th Feb. 1931 .	..	56·4	5·75	0·50	6·25	8·80	8·69	65·3			
17th Feb. 1931 .	..	55·6	5·76	0·69	6·45	8·95	12·01	64·3			
5th Mar. 1931 .	..	56·3	6·31	0·70	7·01	9·29	11·10	68·0			
20th Mar. 1931 .	..	51·3	6·78	0·71	7·49	9·79	10·40	69·3			
6th Apl. 1931 .	..	51·7	6·51	0·59	7·10	9·45	9·04	68·9			
(Average during maximum purity period)	17·4	57·4	7·06	0·27	7·33	9·27	3·83	76·1			

TABLE XXXIV

Showing the effect of frost on Pyrilla *affected Suretha canes (Lehrara Rohtak)*

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks
		Juice	Sucrose	Glucose	Total sugars	Total solids			
7th Nov. 1930 .	..	65·5	4·93	0·36	5·30	7·45	7·33	66·2	Frost as in Table XXXIII
27th Nov. 1930 .	..	65·1	6·15	0·43	6·58	8·48	7·03	72·5	
4th Dec. 1930 .	..	64·5	4·95	0·64	5·59	7·27	13·00	68·2	
17th Dec. 1930 .	..	64·9	4·40	0·60	5·00	7·05	13·57	62·5	
4th Jan. 1931 .	..	62·3	2·74	1·62	4·36	6·78	59·40	40·5	
21st Jan. 1931 .	..	61·9	2·53	1·81	4·34	7·02	71·70	36·1	
7th Feb. 1931 .	..	61·2	3·18	1·44	4·62	6·49	45·20	49·0	
17th Feb. 1931 .	..	59·8	2·46	2·21	4·67	7·53	90·00	32·7	
5th March 1931	..	55·0	2·78	2·33	5·11	6·91	33·80	40·3	
20th Mar. 1931 .	..	55·5	2·98	1·98	4·96	7·65	46·40	39·0	
6th April 1931	..	58·7	3·53	2·26	5·79	8·39	64·20	42·0	
(Average during maximum purity period.)	22·9	65·0	4·61	0·51	5·12	7·56	10·23	67·3	

TABLE XXXV

Showing the effect of frost on Pyrilla *affected Co. 205 canes, (Lehrara Rohtak)*

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks
		Juice	Sucrose	Glucose	Total solids	Total solids			
7th Nov. 1930 .	..	67·1	7·31	0·77	8·08	10·30	10·60	71·0	Frost as in Table XXXIII
21st Nov. 1930 .	..	68·5	7·03	0·62	7·65	9·50	8·81	74·0	
4th Dec. 1930 .	..	61·1	7·18	0·60	7·78	9·64	8·34	74·5	
17th Dec. 1930 .	..	66·2	7·70	0·85	8·55	10·12	11·03	76·1	
4th Jan. 1931 .	..	66·5	7·60	1·10	8·70	10·11	14·40	77·0	
21st Jan. 1931 .	..	68·3	7·47	1·38	8·85	9·79	18·40	76·3	
7th Feb. 1931 .	..	67·7	6·56	1·02	7·58	9·07	15·48	72·3	
17th Feb. 1931 .	..	65·6	6·63	1·13	7·76	9·19	17·01	72·2 72·7	
5th Mar. 1931 .	..	65·0	6·27	1·04	7·31	8·62	16·60		
20th Mar. 1931	..	60·4	5·39	1·04	6·43	8·44	19·20	67·6	
6th Apl. 1931 .	..	61·3	6·06	1·23	7·29	8·97	20·40	67·6	
(Average during maximum purity period.(	15·8	64·3	7·30	0·69	7·99	9·75	9·39	74·8	

TABLE XXXVI

Showing the effect of frost on Co. 213 canes suffering from red rot (Rajah Sansi, Amritsar)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks		
		Juice	Sucrose	Glucose	Total sugars	Total solids					
8th Dec. 1932 .	..	68·5	5·0	1·19	6·2	7·4	23·8	67·5	Frost on	Rain	On
22nd Dec. 1932 .	..	70·8	5·4	1·39	6·8	7·7	25·7	70·1	9-10th Dec. 1932 .	0·70 in.	24th Dec. 1932
5th Jan. 1933 .	..	66·8	5·1	1·38	6·5	7·6	27·0	67·1	16-17th Dec. 1932	0·20 in.	30th Dec. 1932
19th Jan. 1933 .	..	70·2	5·3	1·34	6·6	7·9	25·3	67·1	4th Jan. 1933 .	0·12 in.	18th Jan. 1933
2nd Feb. 1933 .	..	68·3	4·9	1·07	6·0	7·4	21·8	66·2	6th Jan. 1933.	0·44 in.	25th Feb. 1933
16th Feb. 1933 .	..	69·0	4·8	1·20	6·0	7·7	25·0	62·3	8th Jan. 1933.		
7th Mar. 1933 .	..	68·4	5·9	1·19	7·2	7·7	20·2	76·6	10-13th Jan. 1933.		
6th Apl. 1933 .	..	62·1	3·5	0·80	4·3	5·5	22·8	63·6	14-16th Jan. 1933 (H.F.)*		
22nd Dec. 1932 Average during the period of ripeness.	18·7†	70·8	5·4	1·39	6·8	7·7	25·7	70·1	24th-31st Jan. 1933.		
									*H. F.-Heavy frost.		
									†Calculated.		

N

TABLE XXXVII

Showing the effect of frost on Co. 223 canes suffering from red rot (Rajah Sansi, Amritsar)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Fibre per cent	Remarks
		Juice	Sucrose	Glucose	Total sugars	Total solids				
8th Dec. 1932 .	..	63·6	10·0	0·59	10·6	11·7	5·9	84·1	..	
22nd Dec. 1932 .	..	69·2	7·8	1·08	8·8	10·2	13·8	76·5	..	Frost and rain as in Table XXXVI
5th Jan. 1933 .	..	67·7	7·1	1·28	8·4	9·7	18·0	73·2	..	
19th Jan. 1933 .		68·9	6·8	1·05	7·9	9·2	15·4	73·9	13·4	
2nd Feb. 1933 .	..	68·5	5·9	1·30	7·5	8·8	22·0	67·0	..	
16th Feb. 1933 .	..	68·2	5·9	1·25	7·2	8·5	21·2	69·4	..	
7th Mar. 1933 .	..	67·1	7·8	0·67	8·5	9·2	8·6	84·8	..	
6th April 1933 .	..	64·1	6·5	0·64	7·1	8·2	9·8	79·2	..	
8th Dec. 1933 (average during the period of ripeness)	20·0*	63·6	10·0	0·59	10·6	11·7	5·9	84·1	13·4	*Calculated

TABLE XXXVIII

Showing the effect of frost on Co. 300 canes suffering from red rot (Rajah Sansi, Amritsar)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Fibre per cent	Remarks
		Juice	Sucrose	Glucose	Total sugars	Total solids				
8th Dec. 1932 .	..	66·9	5·7	0·91	6·6	7·9	16·0	72·1	..	
22nd Dec. 1932 .	..	65·8	5·3	0·97	6·3	7·6	18·3	69·8	..	Frost and rain as in Table XXXVI
5th Jan. 1933 .	..	70·2	5·1	1·22	6·3	7·9	23·9	64·5		
19th Jan. 1933 .	..	70·0	5·9	1·21	7·1	8·6	21·0	68·6	10·3	
2nd Feb. 1933 .	..	73·4	4·9	1·44	6·3	7·6	29·1	64·8	..	
16th Feb. 1933 .	..	69·6	5·8	1·07	6·9	8·2	18·4	70·7	..	
7th Mar. 1933 .	..	69·2	5·9	0·74	6·6	6·9	12·5	85·5	..	
6th April 1933 .	..	67·9	4·5	1·00	5·5	7·0	22·2	64·3	..	
7th March 1933 (average during the period of ripeness)	19·7*	69·2	5·9	0·74	6·6	7·9	12·5	85·5	10·3	*Calculated

TABLE XXXIX

Showing the effect of frost in the absence of irrigation water (Khudda, Hoshiarpur)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks		
		Juice	Sucrose	Glucose	Total sugars	Total solids					
29th Nov. 1930 .	..	56·9	8·4	0·34	8·7	9·9	4·0	85	Cane attacked by top borer		
11th Dec. 1930 .		58·7	8·1	0·55	8·7	9·9	6·8		Frost on	Rain	on
25th Dec. 1930 .	..	60·2	7·5	0·93	8·4	10·1	12·4	74	8th-12th Dec. 1930 (H. F.)*	0·15 in.	29th Dec. 1930
7th Jan. 1931 .	..	55·2	6·6	0·99	7·6	9·2	15·0	72	19th-21st Dec. 1930	0·11 in.	2nd Feb. 1931
									22nd-27th Dec. 1930 (H. F.)*	0·35 in.	7th Feb. 1931
20th Jan. 1931 .	..	56·5	5·8	1·57	7·4	9·0	27·1	64		0·07 in.	8th Feb. 1931
									1st-2nd Jan. 1931	0·72 in.	17th Feb. 1931
4th Feb. 1931 .	..	56·1	5·7	1·51	7·2	8·8	26·5	65	16th-17th Jan. 1931	0·57 in.	23rd Feb. 1931
										0.10 in.	1st Mar. 1931.
									29th-30th Jan. 1931.	1.08 in.	3rd Mar. 1931
26th Feb. 1931 .	..	51·9	5·6	1·02	6·6	8·0	18·2	70		0·15 in.	4th Mar. 1931
									4th-6th Feb. 1931		
29th Nov. 1930 to 11th Dec. 1930 (average during the maximum purity period)	15·5	57·8	8·3	0·45	8·7	9·9	5·4	84			

*H. F. = Heavy frost.

TABLE XL

Showing the effect of rain alternating with attacks of frost on Katha (Jhabianwala, Jullundur)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks		
		Juice	Sucrose	Glucose	Total sugars	Total solids					
25th Nov. 1930 .	..	59·8	9·8	0·06	9·9	12·1	0·6	81	Cane was slightly attacked by top-borer		
6th Dec. 1930 .	..	61·0	10·3	0·09	10·4	12·2	0·9	84			
21st Dec. 1930 .	..	60·0	9·2	0·11	9·3	11·2	1·2	82	Frost on	Rainfall	on
15th Jan. 1931 .	..	55·0	8·4	0·17	8·6	10·5	2·0	80	8th Dec. 1930 .	0·62 in.	29th Dec. 1930
									9th-12th Dec. 1930	0·17 in.	21st Jan. 1931
28th Jan. 1931 .	..	54·5	8·2	0·21	8·4	10·3	2·6	79	19th-27th Dec. 1930	0·61 in.	24th Jan. 1931
									1st-2nd Jan. 1931	0·84 in.	25th Jan. 1931
10th Feb. 1931 .	..	57·4	8·3	0·20	8·5	10·0	2·4	83	16th-17th Jan. 1931	0·12 in.	3rd Feb. 1931
									29th-30th Jan. 1931	0·09 in.	8th Feb. 1931
1st Mar. 1931 .	..	61·7	8·4	0·22	8·6	10·9	2·2	77		0.02 in.	9th Feb. 1931
										0·50 in.	18th Feb. 1931
11th Mar. 1931 .	..	57·7	9·3	0·10	9·4	11·1	1·1	84		0·32 in.	24th Feb. 1931
										0·03 in.	25th Feb. 1931
25th Mar. 1931 .	..	57·1	8·8	0·45	9·3	11·2	5·1	78		0·31 in.	3rd Mar. 1931
										0·19 in.	4th Mar. 1931
10th Apr. 1931 .	..	59·8	7·6	0·61	8·2	10·5	8·0	72		0·25 in.	5th Mar. 1931
										0·01 in.	16th Mar. 1931
26th Apr. 1931 .	..	58·8	5·6	2·18	7·8	10·2	38·9	55			
25th Nov. 1930 to 11th Mar. 1931 (average during maximum purity period)	13·6	58·4	9·0	0·15	9·1	11·0	1·6	81			

TABLE XLI

Showing the effect of frost in the absence of water on Katha (Jhingar Kalan, Jullundur district)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks
		Juice	Sucrose	Glucose	Total sugars	Total solids			
29th Nov. 1930 .	..	56·2	8·6	0·32	8·9	10·3	3·7	84	Cane was attacked by Top-borer
11th Dec. 1930 .	..	57·2	8·5	0·38	8·9	9·9	4·5	86	Frost same as mentioned in Table XXXIX
25th Dec. 1930 .	..	58·8	7·9	0·75	8·7	10·0	9·5	79	
7th Jan. 1931 .	..	60·0	7·3	1·21	8·5	9·8	16·6	74	
20th Jan. 1931 .	..	54·3	5·7	1·07	6·8	8·0	18·8	71	
4th Feb. 1931 .	..	57·9	6·3	1·16	7·5	9·2	18·4	68	
26th Feb. 1931 .	..	50·4	4·9	1·15	6·1	7·7			Analyses abandoned, canes killed by frost
29th Nov. 1930 to 11th Dec. 1930 (average during maximum purity period)	10·3	56·7	8·6	0·35	8·9				

TABLE XLII

Showing the effect of frost in the absence of water on Co. 205 (Jhingar Kalan, District Jullundur)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks
		Juice	Sucrose	Glucose	Total sugars	Total solids			
29th Nov. 1930 .	..	61·2	7·5	1·20	8·7	9·8	16·0	77	
11th Dec. 1930 .	..	62·6	7·5	1·44	8·9	10·1	19·2	74	Frost same as mentioned in Table XXXIX
25th Dec. 1930 .	..	61·4	5·7	2·51	8·2	9·8	44·0	58	
7th Jan. 1931 .	..	63·7	6·6	2·56	9·2	10·2	38·8	65	
20th Jan. 1931 .	..	57·0	4·9	2·58	7·8	8·8	58·1	56	
4th Feb. 1931 .	..	61·2	5·4	2·53	7·9	9·0	46·8	60	
26th Feb. 1931 .	..	55·6	4·6	2·39	7·0	8·3	51·9	55	Analyses abandoned, canes killed by frosts
29th Nov. 1930 (average during the maximum purity period)	21·0	61·2	7·5	1·20	8·7	9·8	16·0	77	

TABLE XLIII

Showing the effect of frost in the absence of water on Co. 205 Ratoon (Jhingar Kalan, Jullundur district)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks
		Juice	Sucrose	Glucose	Total sugars	Total solids			
29th Nov. 1930 .	..	58·8	7·2	1·22	8·4	10·4	16·9	69	Cane was attacked by rats and Top-borer
11th Dec. 1930 .	..	59·6	7·6	1·78	9·4	11·1	23·4	68	Frost same as mentioned in Table XXXIX
25th Dec. 1930 .	..	62·4	6·8	1·87	8·7	10·8	27·5	63	
7th Jan. 1931 .	..	59·1	5·2	2·98	8·2	9·9	57·2	53	
20th Jan. 1931 .	..	56·4	5·1	2·87	8·0	9·1	56·2	56	
4th Feb. 1931 .	..	56·4	6·1	2·03	8·1	9·8	33·3	63	
26th Feb. 1931 .	..	55·9	4·8	2·14	6·9	9·3	44·5	51	Analyses abandoned, canes killed by frosts
29th Nov. 1930 . (average during maximum purity period)	12·8	58·8	7·2	1·22	8·4	10·4	16·9	69	

TABLE XLIV

Showing the beneficial effect of irrigation in mitigating injury by frost on Co. 223 (Agricultural Station, Jullundur)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks
		Juice	Sucrose	Glucose	Total sugars	Total solids			
26th Nov. 1930 .	..	67·0	9·5	0·68	10·2	11·3	7·2	84	Cane was attacked by *Pyrilla*, Top-borer, and white ant
4th Dec. 1930 .	..	68·1	8·1	0·96	9·1	10·0	11·8	80	Frost and rain same as mentioned in Table XXIX
9th Dec. 1930 .	..	66·8	10·0	0·55	10·6	11·4	5·5	87	
17th Dec. 1930 .	..	69·9	9·0	0·82	9·8	11·0	9·1	82	
14th Jan. 1931 .	..	66·5	8·0	1·17	9·2	10·5	14·6	76	
27th Jan. 1931 .	..	66·5	8·9	0·87	9·8	11·5	9·8	77	
9th Feb. 1931 .	..	65·1	8·4	0·82	9·2	10·6	9·7	79	
25th Feb. 1931 .	..	66·7	8·6	0·89	9·5	10·8	10·3	80	
7th Mar. 1931 .	..	66·7	8·2	0·79	9·0	10·3	9·6	79	
24th Mar. 1931 .	..	65·4	9·2	0·69	9·9	11·1	7·5	83	
9th Apr. 1931 .	..	69·4	9·6	0·63	10·2	11·4	6·5	84	
24th Apr. 1931 .	..	65·7	8·7	1·06	9·8	11·2	12·2	78	
9th Dec. 1930 to 24th Mar. 1931 pr. 1931 (Average during maximum purity period)	24·6	67·2	9·6	0·62	10·2	11·3	6·5	85	

TABLE XLV

Showing the injury to Co. 223 cane by frost in the absence of water (Rasulpur, Hoshiarpur)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks
		Juice	Sucrose	Glucose	Total sugars	Total solids			
30th Nov. 1930 .	..	65·3	8·4	0·79	9·2	10·4	9·4	81	Cane was attacked by Top-borer, white-ants and rats
11th Dec. 1930 .	..	66·2	8·1	1·06	9·2	10·6	13·0	76	Frost and rain same as mentioned in Table XXXIX
25th Dec. 1930 .	..	68·3	8·3	1·18	9·5	10·8	14·2	77	
7th Jan. 1931 .	..	65·1	6·8	2·12	8·9	10·4	31·2	65	
20th Jan. 1931 .	..	65·7	7·1	1·39	8·5	9·7	19·6	73	
4th Feb. 1931 .	..	66·4	7·1	1·04	8·1	9·5	14·6	75	
26th Feb. 1931 .	..	66·9	7·4	0·73	8·1	9·5	9·9	78	
8th Mar. 1931 .	..	67·2	6·9	0·87	7·8	8·9	12·6	77	
18th Mar. 1931 .	..	66·8	6·6	0·67	7·3	8·7	10·0	76	
6th April 1931 .	..	64·3	5·9	0·72	6·6	7·8	12·2	75	
20th April 1931 .	..	61·2	5·1	1·27	6·4	7·6	24·9	67	
30th Nov. 1930 . and 26th Feb. 1931 . (average during maximum purity period)	23·1	66·1	7·9	0·76	8·7	10·0	9·7	80	

TABLE XLVI

Showing the protective action of water against frost on Co. 223 (Rasulpur, Hoshiarpur)

Date of sampling	Weight of stripped cane per acre in tons	Percentage on cane (Calculated from juice)					Glucose ratio	Purity coefficient	Remarks
		Juice	Sucrose	Glucose	Total sugars	Total solids			
30th Nov. 1931 .	..	69·1	8·3	1·39	9·7	10·7	16·7	77	Mild attack of *Pyrilla* and white ants
17th Dec. 1931 .	..	67·9	8·2	1·23	9·4	10·5	15·0	79	Frost on — Rain on
6th Jan. 1932 .	..	68·5	9·2	1·35	10·6	11·6	14·6	79	16th-19th Dec. 1931 0·97 in. 9th Jan. 1932.
22nd Jan. 1932 .	..	68·3	8·3	1·42	9·7	11·0	17·1	75	20th-22nd Dec. 1931 1·55 in. 1st Feb. 1932.
20th Feb. 1932 .	..	65·9	10·5	0·41	10·9	12·0	3·9	87*	26th-28th Dec. 1931 0·25 in. 13th Feb. 1932.
4th Mar. 1932 .	..	62·4	9·9	0·66	10·6	11·6	6·7	85	29th Dec. 1931 (H.F.*) 0·05 in. 10th Mar. 1932.
17th Mar. 1932 .	..	57·9	8·9	0·43	9·4	10·6	5·0	84	31st Dec. 1931 (H·F.*) 0·09 in. 11th Mar. 1932.
20th Feb. 1932 to 17th Mar. 1932 (average during the ripening period)	30·9	62·1	9·8	0·51	10·3	11·4	5·2	85	H. F.—Heavy frost

TABLE XLVII

Showing the number of cases in which different varieties of sugarcane have been classified according to their resistance towards frost (local varieties)

Serial No.	Variety	Resistance to frost	1928-29				1929-30				1930-31				1931-32				1932-33				Total			
			G	J	K	Total	G	J	K	Total	G	J	K	Total	G	J	K	Total	G	J	K	Total	G	J	K	Total
1	Katha .	Resistant .	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	,, .	Semi-resistant.	...	5	...	5	1	5	...	6	1	5	...	6	1	2	...	3	1	1	...	2	4	18	...	22
	,, .	Non-resistant.	...	6	...	6	...	2	...	2	1	2	...	3	...	2	...	2	...	...	...	...	1	12	...	13
		Total .	...	11	...	11	1	7	...	8	2	7	...	9	1	4	...	5	1	1	...	2	5	30	...	35
2	Dhaulu .	Resistant .	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	,, .	Semi-resistant.	...	...	...	...	1	...	...	1	1	...	...	1	2	...	...	2	...	...	...	...	4	...	...	4
	,, .	Non-resistant.	2	...	1	3	1	...	...	1	1	...	...	1	...	...	...	...	...	...	...	...	4	...	1	5
		Total .	2	...	1	3	2	...	...	2	2	...	...	2	2	...	...	2	...	...	...	...	8	...	1	9
3	Kahu .	Resistant .	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	,, .	Semi-resistant.	...	...	...	...	...	...	...	...	1	...	...	1	...	...	...	...	...	...	...	...	1	...	...	1
	,, .	Non-resistant.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
		Total .	...	...	...	...	...	...	...	...	1	...	...	1	...	...	...	...	...	...	...	...	1	...	...	1
4	Yuba .	Resistant .	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	,, .	Semi-resistant.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	,, .	Non-resistant.	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1
		Total .	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1

	Lalri	Resistant.	...	...	2	2	...	...	1	1	...	...	1	1	...	...	...	...	...	...	...	...	...	...	4	4
	„	Semi-resistant.	...	...	1	1	...	...	3	3	...	...	2	2	...	...	...	...	...	...	...	...	...	...	6	6
	„	Non-resistant.	...	...	1	1	...	...	...	...	...	...	1	1	...	...	...	...	...	...	...	...	...	...	2	2
		Total	...	...	4	4	...	...	4	4	...	...	4	4	...	...	...	...	...	...	...	...	...	...	12	12
6	Suretha	Resistant	...	...	3	3	...	...	1	1	...	...	1	1	...	...	...	...	...	...	...	...	...	...	5	5
	„	Semi-resistant.	...	...	...	...	...	...	2	2	...	...	4	4	...	...	...	...	...	...	...	...	...	...	6	6
	„	Non-resistant.	...	...	3	3	...	...	1	1	...	...	1	1	...	...	...	...	...	...	...	...	...	...	5	5
		Total	...	...	6	6	...	...	4	4	...	...	6	6	...	...	...	...	...	...	...	...	...	...	16	16
	Total	Resistant	...	...	5	5	...	...	2	2	...	...	2	2	...	...	...	...	...	...	...	...	...	...	9	9
		Semi-resistant.	...	5	1	6	2	5	5	12	3	5	6	14	3	2	...	5	1	1	...	2	9	18	12	39
		Non-resistant.	3	6	5	14	1	2	1	4	2	2	2	6	...	2	...	2	...	...	...	...	6	12	8	25
		Total	3	11	11	25	3	7	8	18	5	7	10	22	3	4	...	7	1	1	...	2	15	30	29	74

G = Gurdaspur
J = Jullundur
K = Rohtak-Karnal

TABLE XLVIII

Showing the number of cases in which different varieties of sugarcane have been classified according to their resistance towards frost (Coimbatore varieties)

Serial No.	Variety	Resistance to frost.	1928-29				1929-30				1930-31				1931-32				1932-33				Total			
			G	J	K	Total	G	J	K	Total	G	J	K	Total	G	J	K	Total	G	J	K	Total	G	J	K	Total
1	Co. 205 .	Resistant .	...	2	3	5	...	...	2	2	1	...	2	3	...	1	...	1	...	...	...	...	1	3	7	11
	,, .	Semi-resistant.	2	6	3	11	4	7	4	15	2	4	1	7	2	4	...	6	...	2	...	2	10	23	8	41
	,, .	Non-resistant.	1	4	1	6	1	...	...	1	...	3	2	5	...	...	...	...	...	...	...	...	2	7	3	12
		Total .	3	12	7	22	5	7	6	18	3	7	5	15	2	5	...	7	...	2	...	2	13	33	18	64
2	Co. 213 .	Resistant .	1	4	2	7	...	2	2	4	...	1	3	4	1	2	...	3	1	4	1	6	3	13	8	24
	,, .	Semi-resistant.	...	1	1	2	...	6	1	7	...	5	3	8	1	2	...	3	3	4	1	8	4	18	6	28
	,, .	Non-resistant.	...	2	2	4	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1	1	2	2	5
		Total .	1	7	5	13	...	8	3	11	...	6	6	12	2	4	...	6	5	8	2	15	8	33	16	57
3	Co. 223 .	Resistant .	...	2	..	2	...	2	2	4	2	...	1	3	8	1	...	9	...	...	2	2	10	5	5	20
	,, .	Semi-resistant.	2	7	3	12	4	9	2	15	10	6	1	17	4	3	...	7	8	6	...	14	28	31	6	65
	,, .	Non-resistant.	3	2	3	8	1	1	...	2	...	1	...	1	...	...	...	...	...	...	...	...	4	4	3	11
		Total .	5	11	6	22	5	12	4	21	12	7	2	21	12	4	...	16	8	6	2	16	42	40	14	96
4	Co. 270 .	Resistant .	1	...	...	1	1	...	...	1	1	...	...	1	1	...	...	1	...	...	...	...	4	...	...	4
	,, .	Semi-resistant.	...	...	...	...	...	...	...	...	...	...	...	...	...		...	...	1	...	...	1	1	...	...	1
	,, .	Non-resistant.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
		Total .	1	...	...	1	1	...	...	1	1	...	...	1	1	...	...	1	1	...	...	1	5	...	...	5

5	Co. 281 .	Resistant .	1	...	...	1	...	...	...	...	...	1	...	1	...	4	...	4	...	1	...	1	1	6	...	7
	,, .	Semi-resistant.	...	...	...	...	1	...	...	1	...	1	...	1	...	2	...	2	...	...	...	...	1	3	...	4
	,, .	Non-resistant.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
		Total .	1	...	...	1	1	...	...	1	...	2	...	2	...	6	...	6	...	1	...	1	2	9	...	11
6	Co. 285	Resistant .	...	...	2	2	2	1	...	3	1	2	2	5	5	3	...	8	1	4	...	5	9	10	4	23
	,, .	Semi-resistant.	1	...	...	1	3	...	1	4	5	...	...	5	...	4	...	4	14	1	...	15	23	5	1	29
	,, .	Non resistant.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
		Total .	1	...	2	3	5	1	1	7	6	2	2	10	5	7	...	12	15	5	...	20	32	15	5	52
7	Co. 290 .	Resistant .	...		...	...	...	1	...	1	2	...	1	3	2	2	...	4	...	...	...	...	4	3	1	8
	,, .	Semi-resistant.	1	...	...	1	2	...	1	3	1	1	...	2	1	3	...	4	6	...	...	6	11	4	1	16
	,, .	Non-resistant.	...	...	2	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	2
		Total .	1	...	2	3	2	1	1	4	3	1	1	5	3	5	...	8	6	...	...	6	15	7	4	26
	Total .	Resistant .	3	8	7	17	3	6	6	15	7	4	9	20	17	13	...	30	3	9	3	15	33	40	25	97
	,, .	Semi-resistant.	6	14	7	27	14	22	9	45	18	17	5	40	8	18	...	26	31	13	1	45	77	84	22	183
	,, .	Non-resistant.	4	8	8	20	2	1	...	3	...	4	2	6	...	...	...	...	1	...	...	1	7	13	10	30
		Total .	13	30	22	64	19	29	15	63	25	25	16	66	25	31	...	56	35	22	4	61	117	137	57	310

G = Gurdaspur J = Jullundur K = Rohtak and Karnal

TABLE XLIX

Effect of Pyrallid borer (Co. 205)

Date of sampling	Description	Percentage on cane				Glucose ratio	Purity coefficient
		Juice	Sucrose	Glucose	Total solids		
15th Dec. 1931 .	Healthy . .	61·9	7·7	0·82	10·9	10·7	75·2
	Diseased . .	59·1	4·9	2·13	9·3	43·5	52·7
21st Jan. 1932 .	Healthy . .	62·1	8·5	0·61	10·9	7·2	78·0
	Diseased . .	62·6	4·9	2·80	10·0	57·1	48·0

TABLE L

Effect of Pyrilla

Yield in tons per acre

Variety	Year	Stripped cane	Total solids	Sucrose	Glucose
Lalri	1929-30*	15·7	1·70	1·38	0·019
	1930-31	16·4	1·61	1·27	0·034
Suretha	1929-30	24·0	2·64	2·13	0·037
	1930-31	19·6	1·97	1·53	0·062
Co. 205	1929-30	22·9	2·50	2·09	0·042
	1930-31	18·6	1·90	1·57	0·074
Co. 213	1929-30	24·3	2·89	2·56	0·019
	1930-31	22·0	2·22	1·84	0·080

*1929-30 crop was practically free from Pyrilla, while in 1930-31 it was severely attacked.

TABLE LI

Data from free and Pyrilla affected neighbouring canes (per cent on cane)

Variety	Juice	Total solid	Sucrose	Glucose	Glucose ratio	Purity coefficient	Remarks
1. Co. 213. .	68·7	9·8	7·4	0·74	10·0	75	Pyrilla-affected
2. ,, .	66·8	12·5	10·9	0·18	1·6	87	,, free
3. Co. 223. .	74·2	12·0	10·1	0·50	4·9	84	,, —affected*
4. ,, . .	67·2	11·3	9·6	0·62	6·5	85	,, —free*
5. Co. 281. .	69·6	11·5	9·5	0·24	2·6	82	,, —affected
6. ,, . .	67·6	13·6	11·9	0·20	1·7	88	,, —free
7. Co.-285. .	65·1	11·2	8·8	0·24	2·7	79	,, —affected
8. ,, . .	63·3	11·9	10·0	0·14	1·3	84	,, —free
Mean value .	67·8	10·8	8·6	0·41	4·8	80	,, —affected
	65·9	12·7	10·9	0·17	1·6	86	,, —free

*Omitted from calculation from mean value as this variety was severely attacked by Pyrilla even in the Pyrilla-free fields.

TABLE LII

Showing that "mosaic" did not affect the composition of the cane

Date of sampling	Variety	Description	Juice	Percentage of cane			Glucose ratio	Purity coefficient
				Total solids	Sucrose	Glucose		
10th Dec. 1929 .	Co. 223 .	Healthy . .	63·1	10·3	9·4	0·6	6·7	91·0
	,, .	Diseased . .	63·6	10·3	9·3	0·6	6·4	90·0
1st Jan. 1931 .	,, .	Healthy . .	65·1	10·7	7·7	1·8	23·3	71·9
	,, .	Diseased . .	65·0	10·9	7·9	1·7	21·5	72·4
2nd Feb. 1931 .	,, .	Healthy . .	68·2	11·6	8·9	1·5	16·8	76·7
	,, .	Diseased . .	69·0	11·7	8·7	1·6	18·4	74·3
12th Jan. 1932 .	,, .	Healthy . .	..	10·1	8·5	0·67	7·9	84·2
	,, .	Diseased . .	..	10·2	8·6	0·68	7·9	84·3

TABLE LIII

Before or soon after the attack of "red rot"

Date of sampling	Variety	Percentage on cane				Glucose ratio	Purity coefficient
		Juice	Total solids	Sucrose	Glucose		
			Before attack				
8th Dec. 1932 . .	Co. 213 .	68·5	7·4	5·0	1·19	23·8	67·5
	Co.-223 .	63·6	11·7	10·0	0·59	5·9	84·1
	Co.-285 .	61·6	10·9	9·3	0·48	5·1	85·3
	Co. 290 .	68·2	12·4	10·6	0·35	3·3	85·0
	Co. 300 .	66·9	7·9	5·7	0·91	16·0	72·1
			Two months after the attack				
2nd Feb. 1933 . .	Co. 213 .	68·3	7·4	4·9	1·07	21·8	66·2
	Co. 223 .	68·5	8·8	5·9	1·30	22·0	67·0
	Co. 285 .	63·1	9·8	7·8	0·49	6·3	79·6
	Co. 290 .	68·0	10·1	7·2	0·77	10·6	72·0
	Co. 300 .	73·4	7·6	4·9	1·44	29·1	64·8

TABLE LIV

Comparative data on flowering and flowerless canes

Serial No.	Date of sampling	Locality	Average weight per cane in grms.		Juice per cent on cane		In flowers					Flowerless				
			In flowers	Flowerless	In flowers	Flowerless	Sucrose	Glucose	Total solids	Glucose ratio	Purity coefficient	Sucrose	Glucose	Total solids	Glucose ratio	Purity coefficient
							Co. 205.									
1	29th Jan. 1932.	Agri. Farm, Gurdaspur	607 (17)*	508(19)*	58·1	60·8	15·5	0·57	18·4	3·7	84	15·5	0·61	18·6	3·9	83
2	12th Feb. 1932	„ „ „	714 (8)	573(12)	59·7	63·1	15·1	0·50	17·8	3·3	85	13·3	0·75	17·0	5·6	79
3	28th Feb. 1932	„ „ „	766 (5)	448(10)	62·3	59·3	16·9	0·33	19·1	2·0	89	14·7	0·52	17·7	3·6	83
4	1st Mar. 1932 .	„ „ „	745 (9)	519(11)	59·2	62·6	15·6	0·42	18·8	2·7	83	18·6	0·75	16·8	5·5	81
5	23rd Mar. 1932	„ „ „	732 (8)	358(10)	58·9	...	16·9	0·37	19·9	2·2	85	15·6	0·52	18·5	3·3	84
6	22nd Feb. 1932.	Sargondi (Jullundur) .	495(10)	488(10)	56·4	60·0	16·2	0·72	19·1	4·5	85	16·0	1·00	19·1	6·5	82
7	29th Feb. 1932	Kot Kalan (Jullundur)	608(6)	445 (6)	53·7	57·4	16·2	1·06	18·9	6·5	86	14·7	1·41	18·3	9·5	81
8	7th Mar. 1932 .	Sargondi („)	413(12)	320(12)	53·3	54·6	19·1	0·80	22·2	4·2	86	17·2	0·92	21·1	5·3	82
																Ratoon.
9	18th Mar. 1932	Attalgarh (Hoshiarpur)	440(10)	286(10)	57·6	...	...	...	...	...	...	...	...	...	...	...
10	20th Mar. 1932	Machiwara (Ludhiana)	480(10)	409(10)	57·9	55·7	17·6	0·69	20·7	3·9	83	18·8	0·55	21·2	3·2	81
		Average. .	580	442	57·9	59·2	16·6	0·61	19·5	3·7	85	15·5	0·78	18·5	5·0	83
							Co. 285.									
11	29th Jan. 1931.	Agri. Farm, Gurdaspur	830(17)	562(18)	65·6	65·7	15·9	0·61	18·0	3·8	88	15·2	0·76	17·5	5·0	87
12	19th Feb. 1931	„ „ „	756(20)	647(17)	64·6	66·0	16·3	0·46	18·3	2·8	89	15·5	0·61	17·9	3·9	86
13	13th Mar. 1931	„ „ „	725(20)	472(20)	64·4	64·9	16·6	0·31	19·1	1·9	87	16·3	0·31	18·6	1·9	88
14	23rd Feb. 1932	Jabbowal (Jullundur)	880 (9)	638 (8)	59·5	61·8	17·2	0·40	19·1	3·4	89	16·0	0·33	18·0	2·1	89
15	9th Mar. 1932 .	„ „ .	944 (9)	750(9)	63·5	63·3	17·7	0·26	20·0	1·4	88	17·3	0·35	19·8	2·0	88
16	18th Mar. 1932	Attalgarh (Hoshiarpur)	875 (8)	769 (8)	57·8	56·5	17·1	0·18	19·8	1·1	87	17·0	0·19	19·5	1·1	87
17	20th Mar. 1932	Lalaudi (Ludhiana) .	753(10)	689(10)	55·9	57·1	18·0	0·31	21·0	1·7	86	18·2	0·26	21·1	1·4	87
		Average .	803	616	61·6	62·2	17·0	0·36	19·3	2·1	88	16·5	0·40	18·9	2·4	87

Figures within brackets indicate the number of individual canes weighed to get the average weight of one cane.

TABLE LV

Rainfall in inches during five years in the Gurdaspur and Jullundur districts during the sugarcane seasons 1927-28 to 1931-32

	March	April	May	June	July	August	September	October	November	December	January	February	Total
1927-28 G	1·35	0·25	2·33	0·81	10·08	6·34	0·77	...	0·05	0·67	2·67	1·19	26·51
„ J	0·72	0·10	0·57	1·90	11·14	3·93	0·18	...	0·10	1·22	2·29	2·64	24·79
1928-29 G	0·39	0·12	0·18	0·90	4·07	9·71	3·50	0·18	2·85	2·59	0·74	0·30	26·63
„ J	0·10	0·28	0·20	1·83	1·63	3·10	2·17	...	0·76	1·32	0·50	0·10	11·99
1929-30 G	..	..	0·22	3·04	10·50	4·67	0·87	3·68	..	5·93	1·98	1·04	31·93
„ J	..	0·41	..	2·47	9·64	3·45	1·60	1·11	..	2·19	0·90	1·00	22·77
1930-31 G	1·08	1·47	0·05	3·39	15·45	4·95	2·53	0·08	..	0·05	0·72	2·53	32·30
„ J	0·31	0·40	..	2·42	10·31	3·34	6·09	..	..	0·54	1·52	0·98	25·97
1931-32 G	0·96	0·45	1·21	0·60	4·42	14·6	1·08	0·63	..	..	1·52	1·77	27·14
„ J	0·75	0·28	1·50	0·16	6·21	13·4	0·38	1·16	..	0·02	0·44	0·70	25·00
						1927	1928	1929	1930	1931			
Total for September, October, November and December—G						1·49	9·12	10·48	2·66	1·71*			
J						1·50	4·25	4·90	6·63	1·56*			

NOTE.—'G' stands for Gurdaspur and 'J' for Jullundur.

* 2·66 inches of rain at Gurdaspur and 1·08 in. at Jullundur, recorded on 1st September 1931, are not included as they relate to rain received on 31st August 1931

TABLE LVI

Yields in maunds of gur *per acre from plant and its ratoon crops*

Year	Co. 213	Co. 223	Remarks
1928-29 . . .	59·58	56·80	Plant crop
1929-30 . . .	44·80	52·40	First ratoon
1930-31 . . .	45·75	53·85	Second ratoon
1931-32 . . .	42·40	52·10	Third ratoon
1932-33 . . .	36·00	53·90	Fourth ratoon

TABLE LVII

Comparative data for plant and ratoon crop

Variety	Year	Weight in tons per acre				Percentage on stripped cane				Purity coefficient.
		Stripped cane	Sucrose	Glucose	Total solids	Juice	Sucrose	Glucose	Fibre	
Co. 205 .	1931-32 (Plant crop) . .	13·6	1·22	0·078	1·66	59·0	9·0	0·57	..	78
„	1932-33 (Ratoon crop) . .	17·2	1·56	0·117	1·94	59·1	9·1	0·68	20·8	81
Co. 285 .	1931-32 (Plant crop) . .	18·7	1·80	0·045	2·13	59·6	9·6	0·24	..	84
„ .	1932-33 (Ratoon crop) . .	20·2	1·94	0·038	2·32	62·8	9·6	0·19	16·1	83

TABLE LVIII

Fibre percentage on cane

	Jullundur	Gurdaspur
Katha	18·1	21·7
Co. 205	18·5	..
Co. 213	14·5	12·7
Co. 223	11·8	14·5
Co. 270	..	17·2
Co. 281	13·9	17·2
Co. 285	16·5	17·7
Co. 290	12·2	12·0
Co. 300	14·5	12·2
Co. 301	14·0	..
Co. 312	13·0	..
Co. 313	15·0	..

TABLE LIX

Showing results from water-withholding experiments (1932-33)

Locality	Weight of stripped cane in tons per acre	Percentage on cane				Glucose ratio	Purity coefficient
		Juice	Total solids	Sucrose	Glucose		
		Normal watering					
		Co. 213					
Jullundur Agricultural Station	20·7	67·4	12·9	11·3	0·28	2·6	88
Jallowal	18·0	65·6	12·3	10·8	0·33	3·1	88
Baiyanpur (Sonepat)	27·3	68·8	11·7	9·9	0·46	4·7	85
Average	22·0	67·3	12·3	10·7	0·36		
		Co. 223					
Jullundur Agricultural Station	24·2	65·9	11·6	10·1	0·41	4·1	87
Jallowal	15·6	63·7	11·7	10·3	0·55	5·3	88
Agricultural Farm, Hansi	23·2	63·7	11·6	10·3	0·38	3·8	88
Average	21·0	64·4	11·6	10·2	0·45		
		Water withheld from 1st October onwards					
		Co. 213					
Jullundur Agricultural Station	20·3	67·7	13·0	11·4	0·39	3·6	87
Jallowal	14·2	65·5	12·2	10·7	0·40	3·8	88
Baiyanpur (Sonepat)	23·5	69·8	12·1	10·1	0·52	5·1	83
Average	19·3	67·7	12·4	10·73	0·44		
		Co. 223					
Jullundur Agricultural Station	18·9	66·4	11·7	10·1	0·44	4·4	87
Jallowal	14·8	63·3	11·7	10·3	0·54	5·3	88
Agricultural Farm, Hansi	17·7	64·4	12·4	11·0	0·40	3·6	89
Average	17·1	64·7	11·9	10·5	0·46		

TABLE LX

Showing effect of artificial manures on Co. 223 plant crop

Manure applied	Weight in tons per acre				Percentage on cane			
	Stripped cane	Sucrose	Glucose	Total solids	Juice	Sucrose	Glucose	Total solids
Potassium nitrate . .	32·23	3·36	0·157	3·76	67·4	10·4	0·49	11·7
Superphosphate . .	29·23	2·97	0·141	3·38	67·7	10·2	0·49	11·6
Ammonium sulphate . .	31·78	3·24	0·165	3·69	66·9	10·2	0·52	11·6
Ammonium phosphate .	28·97	2·97	0·152	3·33	67·4	10·3	0·52	11·5
Control	29·07	2·90	0·153	3·29	66·7	10·0	0·52	11·4

TABLE LXI

Showing effect of artificial manures on Co. 285 plant crop

Manure applied	Weight in tons per acre				Percentage on cane			
	Stripped cane	Sucrose	Glucose	Total solids	Juice	Sucrose	Glucose	Total solids
Potassium nitrate . .	27·4	2·68	0·093	3·04	61·2	9·8	0·34	11·1
Superphosphate . .	24·0	2·40	0·067	2·74	61·2	10·0	0·28	11·4
Ammonium sulphate . .	28·4	2·90	0·071	3·27	60·9	10·2	0·25	11·5
Ammonium phosphate .	27·2	2·77	0·075	3·10	60·5	10·2	0·27	11·4
Control	21·4	2·14	0·051	2·33	59·2	10·0	0·24	10·9

TABLE LXII

Yield of stripped cane in tons per acre (1927-28 to 1932-33)

Year	Circle	Lalri	Suretha	Dhaulu	Katha	Co. 205	Co. 213	Co. 223	Co. 270	Co. 281	Co. 285	Co. 290	Co. 300	Co. 301	Co. 312	Co. 313	Average of local varieties	Average of Co. varieties
1927-28	Gurdaspur	...	...	12·1	...	17·6	18·2	20·2	...	...	...	...	...	...	...	...	12·1	18·7
,,	Jullundur	...	...	24·6	25·1	28·6	...	30·5	...	...	...	...	...	...	...	...	24·8	29·5
,,	Rohtak-Karnal	29·0	42·8	26·0	...	28·4	35·3	26·9	...	...	...	...	...	...	...	...	32·6	30·2
																	23·2	26·2
1928-29	Gurdaspur	...	...	8·5	...	13·0	22·5	20·3	26·9	20·4	30·0	27·0	...	...	...	...	8·5	22·6
,,	Jullundur	...	...	...	12·2	16·8	13·3	17·9	...	...	...	...	...	...	...	...	12·2	16·0
,,	Rohtak-Karnal	20·7	24·8	25·2	...	26·9	28·5	30·6	...	...	...	...	...	...	...	...	23·6	28·7
																	14·8	22·4
1929-30	Gurdaspur	...	...	11·6	11·2	16·9	...	19·1	21·4	12·4	15·8	16·7	...	...	...	...	11·4	17·0
,,	Jullundur	...	...	...	14·4	23·8	25·9	22·1	...	...	28·3	23·3	...	...	...	...	14·4	24·7
,,	Rohtak-Karnal	15·7	24·0	...	...	22·9	24·3	23·7	...	...	...	...	...	...	...	...	19·9	23·6
																	15·2	21·8
1930-31	Gurdaspur	...	...	15·4	13·7	22·7	...	17·6	20·3	...	20·0	17·3	...	...	...	...	14·5	19·6
,,	Jullundur	...	...	...	12·9	20·2	21·2	23·3	...	22·8	27·8	27·4	...	...	...	...	12·9	20·4
,,	Rohtak-Karnal	16·4	19·6	...	...	18·6	22·0	...	...	...	...	...	...	...	...	...	18·0	20·3
																	15·1	20·1
1931-32	Gurdaspur	...	...	...	9·0	17·4	17·8	16·0	13·6	...	21·5	20·3	...	...	...	...	9·0	17·2
,,	Jullundur	...	...	...	16·1	23·9	30·2	25·8	...	19·4	30·1	27·3	...	...	...	...	16·1	26·1
																	12·5	21·6
1932-33	Gurdaspur	...	...	...	12·7	...	17·1	17·6	21·5	...	22·2	21·3	22·7	...	...	...	12·7	20·4
,,	Jullundur	...	...	...	15·7	21·5	21·4	18·0	...	23·2	26·7	24·4	27·2	25·2	29·8	23·3	15·7	24·1
																	14·2	22·2
Average	Gurdaspur	...	...	11·9	11·6	17·5	18·9	18·4	17·3	16·4	21·9	20·5	22·7	...	...	...		
	Jullundur	...	...	...	16·1	22·5	28·0	22·8	...	21·8	28·2	25·6	27·2	25·2	29·8	23·3		
	Rohtak-Karnal	20·5	27·8	25·6	...	24·2	27·5	27·1	...	...	...	...	...	...	...	...		

TABLE LXIII

Amounts of sucrose in tons per acre (1927-28 to 1932-33).

Year	Circle	Lalri	Suretha	Dhaulu	Katha	Co. 205	Co. 213	Co. 223	Co. 270	Co. 281	Co. 285	Co. 290	Co. 300	Co. 301	Co. 312	Co. 313	Average local varieties	Average coimbatore varieties
1927-28	Gurdaspur	...	...	0·98	...	1·49	1·77	1·86	...	...	...	...	...	...	...	...	0·98	1·71
,,	Jullundur	...	...	2·22	2·59	2·71	...	3·15	...	...	...	...	...	...	...	...	2·41	2·93
,,	Rohtak-Karnal	2·25	3·84	2·36	...	2·45	3·72	2·79	...	...	...	...	...	...	...	...	2·82	2·99
																	2·07	2·54
1928-29	Gurdaspur	...	...	0·68	...	1·01	2·11	1·76	2·78	2·31	2·76	2·89	...	...	...	...	0·68	2·23
,,	Jullundur	...	...	...	1·04	1·38	1·12	1·40	...	...	...	...	...	...	...	...	1·04	1·30
,,	Rohtak-Karnal	1·53	2·00	1·82	...	2·10	2·33	2·23	...	...	...	...	...	...	...	...	1·17	1·92
																	1·17	1·92
1929-30	Gurdaspur	...	...	0·93	1·02	1·40	...	1·44	1·89	1·19	1·52	1·61	...	...	...	...	0·98	1·51
,,	Jullundur	...	...	...	1·24	2·28	2·88	2·10	...	...	2·56	2·11	...	...	...	...	1·24	2·39
,,	Rohtak-Karnal	1·38	2·13	...	...	2·09	2·56	2·35	...	...	...	...	...	...	...	..	1.76	2·33
																	1·33	2·08
1930-31	Gurdaspur	...	...	1·29	1·25	2·15	...	1·66	2·45	...	2·02	2·03	...	...	...	...	1·27	2·06
,,	Jullundur	...	...	...	1·10	1·82	2·12	2·21	...	2·41	2·59	2·31	...	...	...	...	1·10	2·24
,,	Rohtak-Karnal	1·27	1·53	...	...	1·57	1·94	...	...	...	...	...	...	...	...	...	1·40	1·71
																	1·26	2·00
1931-32	Gurdaspur	...	...	1·08	0·83	1·64	1·82	1·50	1·22	...	2·07	2·17	...	...	...	...	0·96	1·74
,,	Jullundur	...	...	...	1·42	2·15	3·29	2·48	...	2·23	2·93	3·01	...	...	...	...	1·42	2·68
																	1·19	2·21
1932-33	Gurdaspur	...	...	...	1·27	...	1·79	1·69	2·19	...	2·18	2·31	2·38	...	...	...	1·27	2·09
,,	Jullundur	...	...	...	1·38	2·00	2·29	1·82	...	2·81	2·66	2·98	3·07	2·85	3·22	2·74	1·38	2·64
																	1·33	2·37
Average	Gurdaspur	...	...	0·93	1·09	1·52	1·87	1·65	2·11	1·75	2·11	2·20	2·38	...	...	...		
	Jullundur	...	...	2·22	1·46	2·06	2·34	2·19	...	2·48	2·69	2·60	3·07	2·85	3·22	2·74		
	Rohtak-Karnal	1·61	2·38	2·36	...	2·05	2·61	2·46	...	...	...	...	...	...	...	...		

TABLE LXIV

Average outturn in tons per acre (1927-28 to 1932-33).

Name of circle	Stripped cane		Sucrose	
	Local	Coimbatore	Local	Coimbatore
Gurdaspur	11·2	19·6	1·04	1·95
Jullundur	17·3	24·1	1·57	2·62
*Rohtak-Karnal	24·60	26·67	1·83	2·35

*From year 1927-28 to 1930-31.

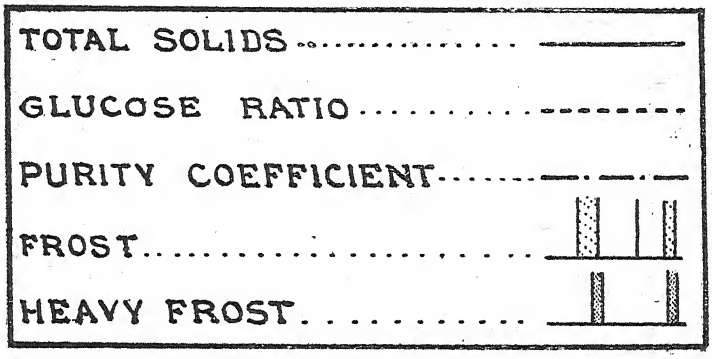
TABLE LXV

Area (in acres) under sugarcane in the Punjab

District	1927-28	1928-29	1929-30	1930-31	1931-32	1932-33	Average of the six years
Hissar . . .	1,208	451	211	568	1,062	1,705	866
Rohtak . . .	41,369	29,034	15,273	35,422	27,713	37,889	31,117
Gurgaon . . .	10,636	6,455	3,889	9,505	11,710	12,230	9,071
Karnal . . .	33,932	21,287	21,856	29,839	25,611	32,180	27,451
Amballa . . .	17,817	16,861	12,460	13,463	17,040	24,587	17,038
Simla . . .	1	..	..	..	..	..	..
Kangra . . .	5,561	5,094	2,293	3,119	4,259	4,762	4,181
Hoshiarpur . .	28,818	25,551	14,609	21,076	26,467	34,053	25,096
Jullundur . . .	32,240	26,194	19,959	27,644	31,638	37,095	29,128
Ludhiana . . .	15,449	11,676	9,578	11,382	14,125	18,870	13,513
Ferozepur . . .	4,823	3,096	1,906	3,993	5,422	10,634	4,979
Lahore . . .	19,190	13,786	7,590	14,819	25,360	28,540	18,214
Amritsar . . .	32,405	24,002	17,468	22,065	25,758	32,519	25,703
Gurdaspur . . .	61,624	54,418	38,131	49,613	58,397	69,584	55,295
Sialkot . . .	40,874	36,038	30,987	34,795	32,820	37,628	35,524

District	1927-28	1928-29	1929-30	1930-31	1931-32	1932-33	Average of the six years
Gujranwala . .	25,244	25,124	25,092	23,248	21,806	25,430	24,324
Sheikhupura . .	20,171	16,739	17,068	19,804	19,760	22,223	19,294
Gujrat . . .	12,904	12,140	10,798	9,303	8,964	13,376	11,248
Shahpur . . .	8,816	8,144	8,472	8,559	8,512	9,547	8,675
Jhelum . . .	217	175	120	128	190	252	180
Rawalpindi. . .	79	87	63	62	73	86	74
Attack . . .	781	674	665	777	820	834	759
Mianwali . . .	76	80	42	14	29	67	51
Montgomery . .	15,000	10,007	6,176	13,138	19,801	19,775	13,983
Lyallpur . . .	51,445	39,082	30,946	55,762	63,551	55,475	49,377
Jhang . . .	2,847	2,683	3,043	4,300	4,704	4,482	3,677
Multan . . .	8,712	6,742	4,259	8,928	12,953	15,594	9,531
Muzaffargarh . .	6,319	4,880	3,548	4,001	5,983	8,567	5,550
Dera Ghazi Khan .	66	104	104	102	127	168	112
Total .	498,624	400,904	306,696	425,729	474,555	558,152	447,011

References to Figs. 1—16



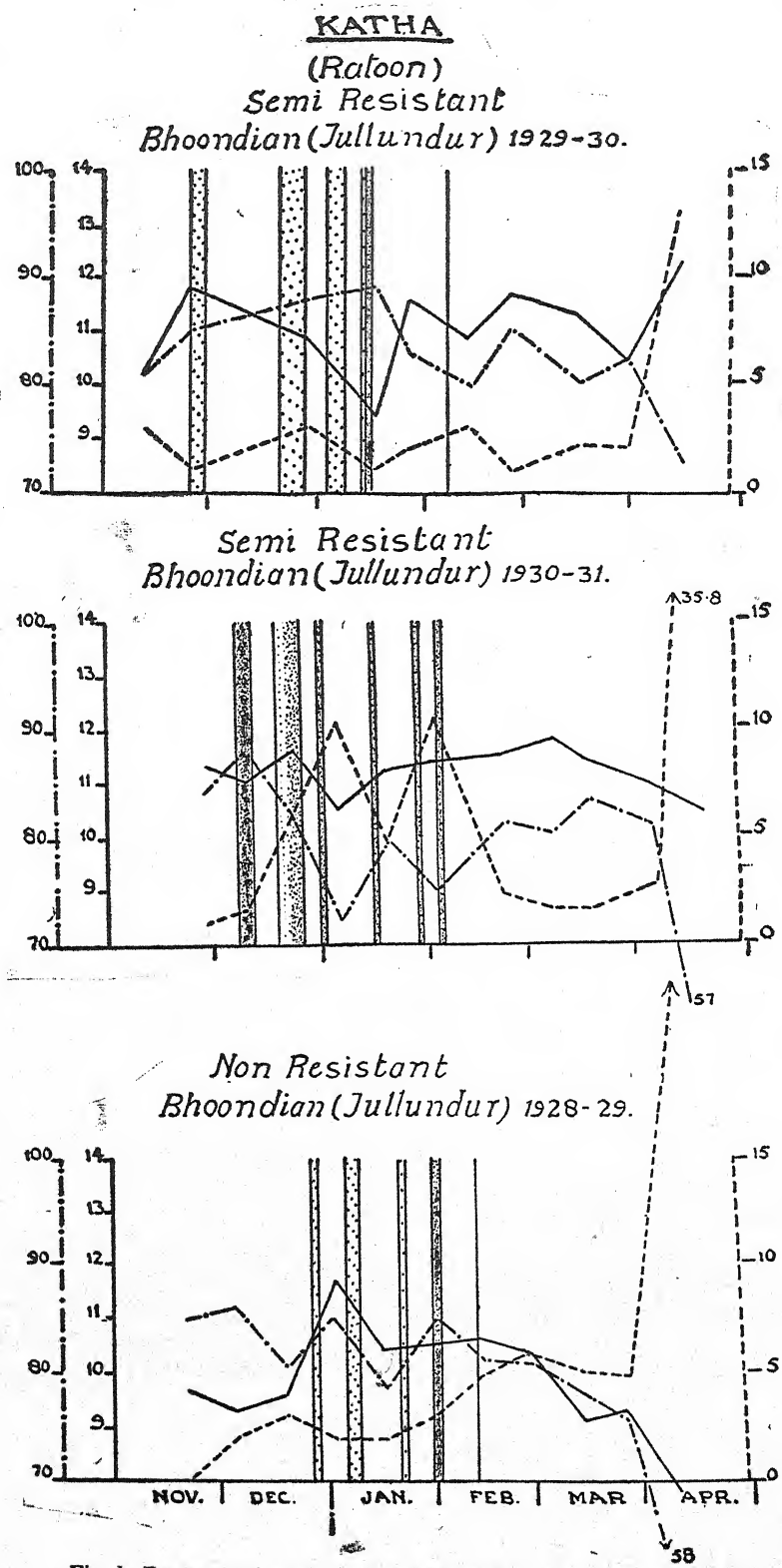


Fig. 1. Response of Katha to frost as a semi and non-resistant can

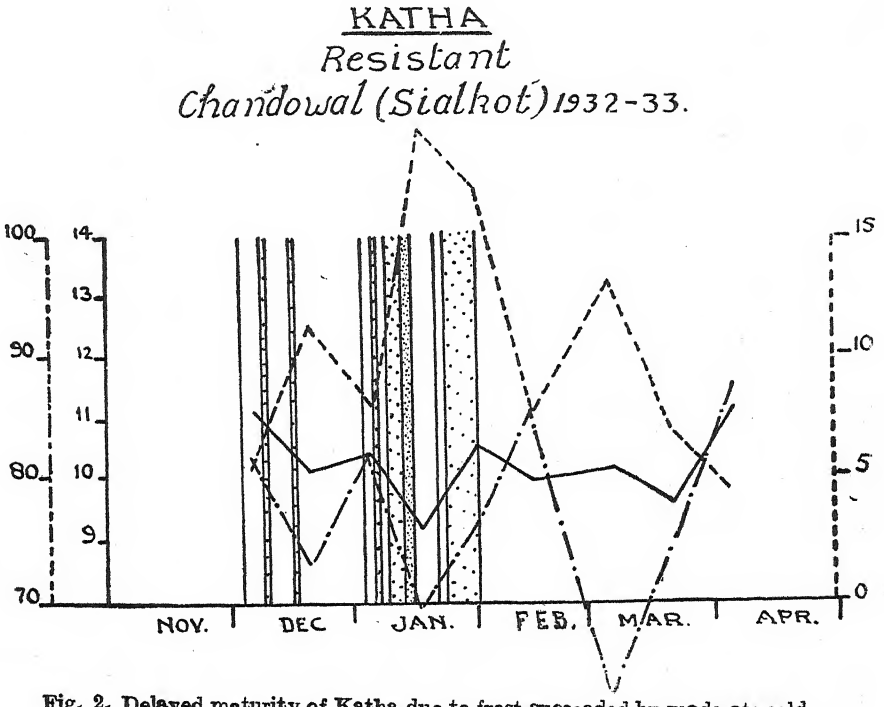


Fig. 2. Delayed maturity of Katha due to frost succeeded by moderate cold

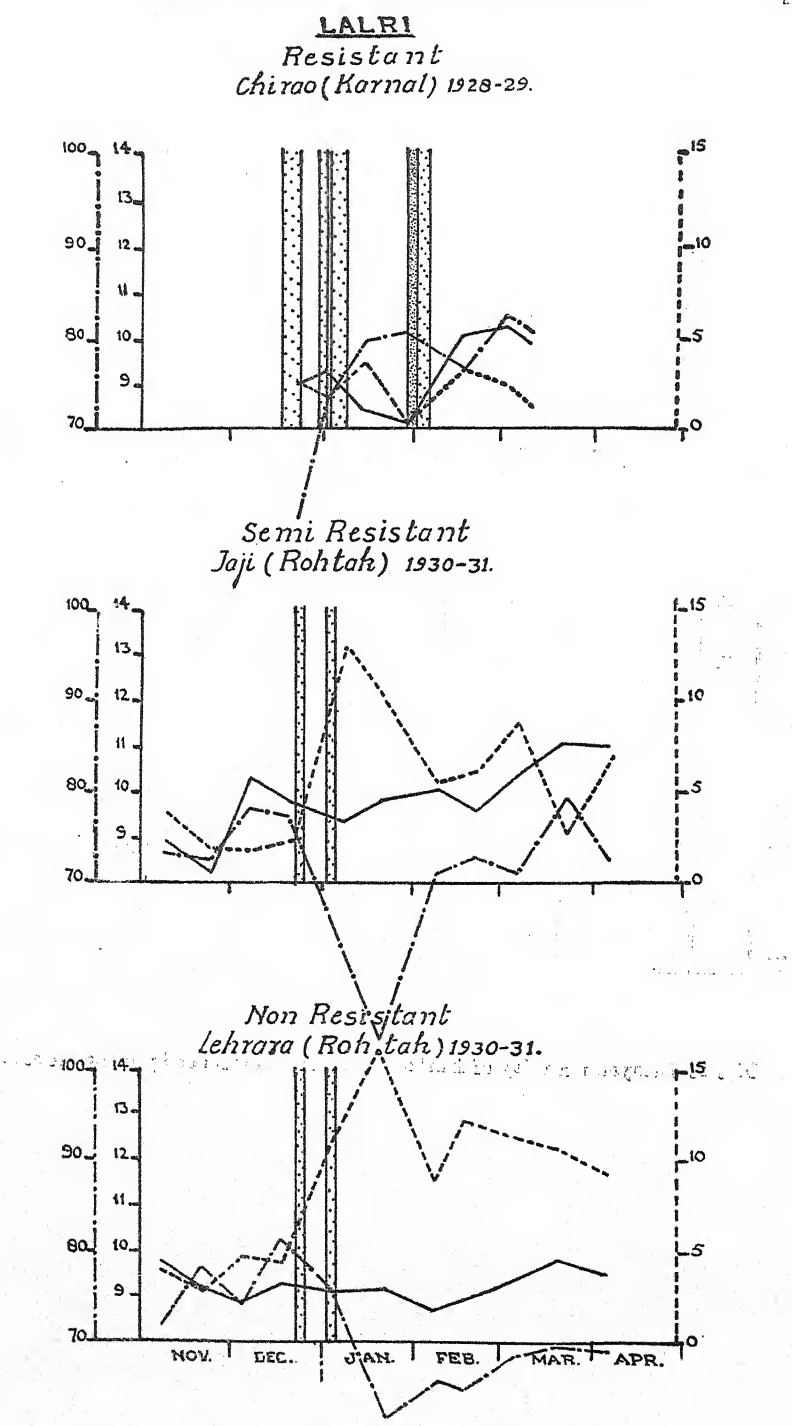


Fig. 3. Response of Lalri to frost as a resistant, semi and non-resistant cane

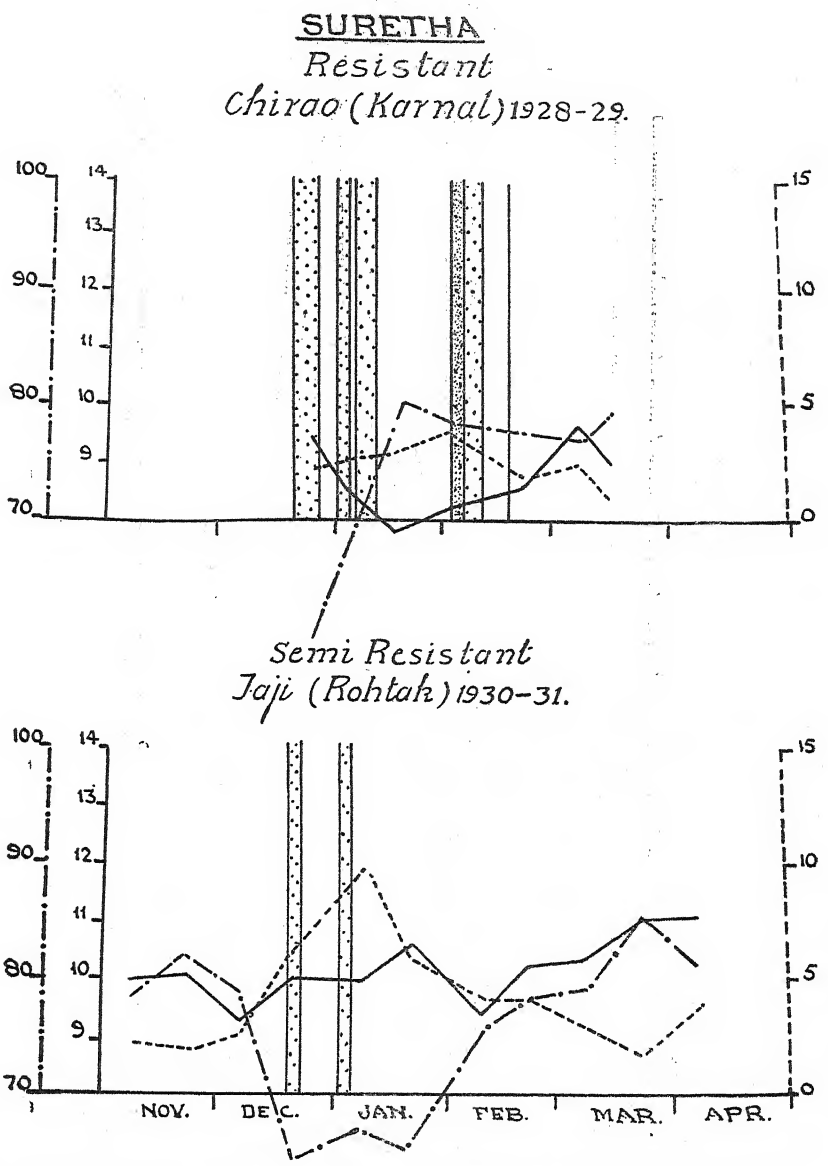


Fig. 4. Response of Suretha to frost as a resistant, and semi-resistant cane

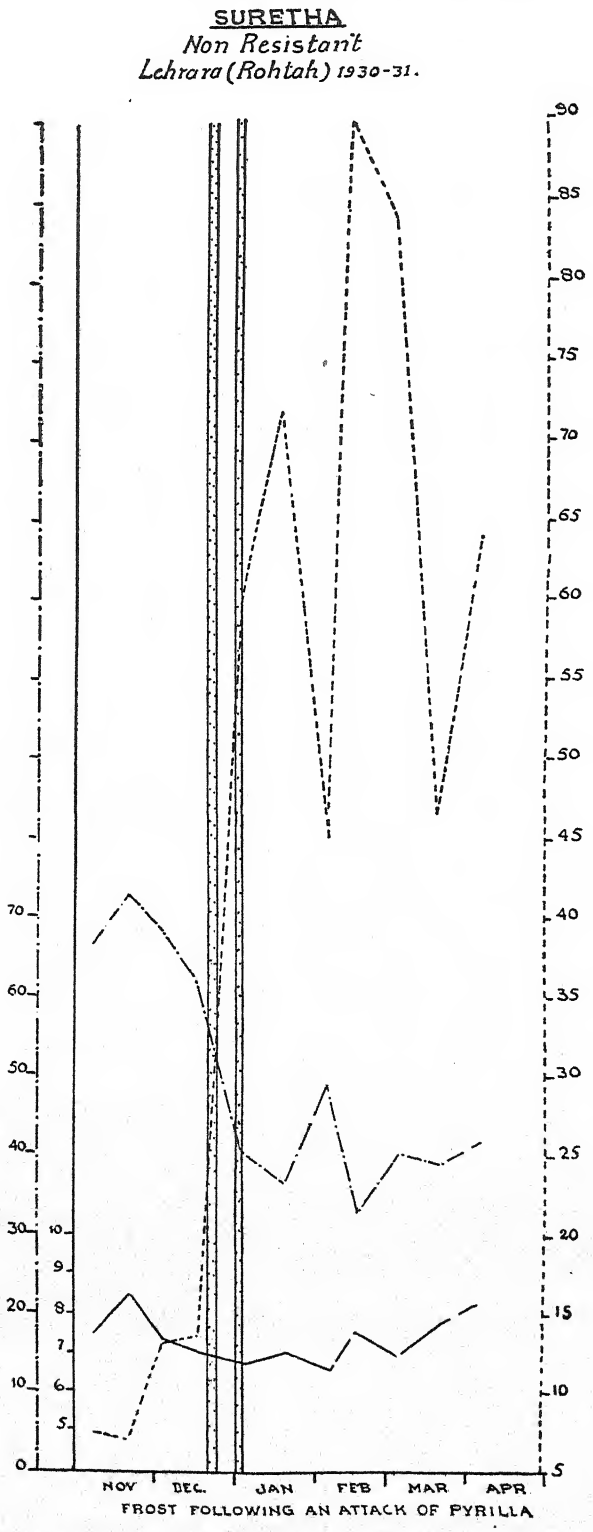


Fig. 5. Nonresistant Suretha, injury by Pyrilla intensified by following frost

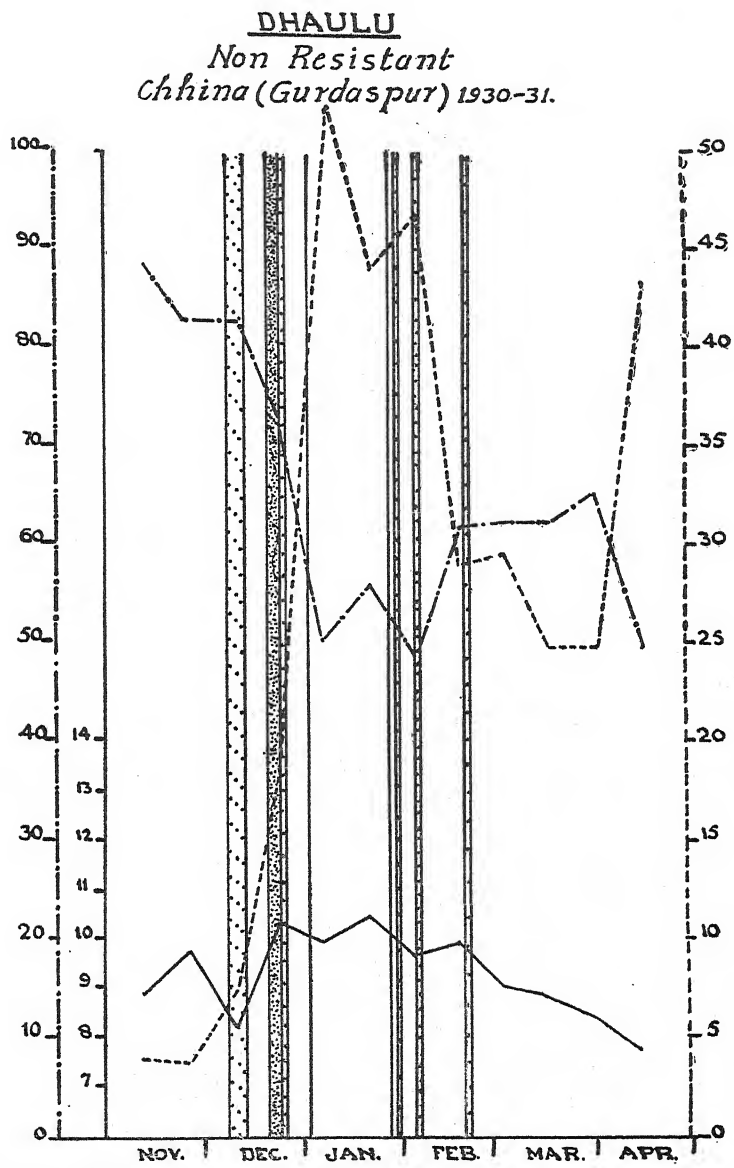


Fig. 6. Great susceptibility of Dhaulu to injury by frost

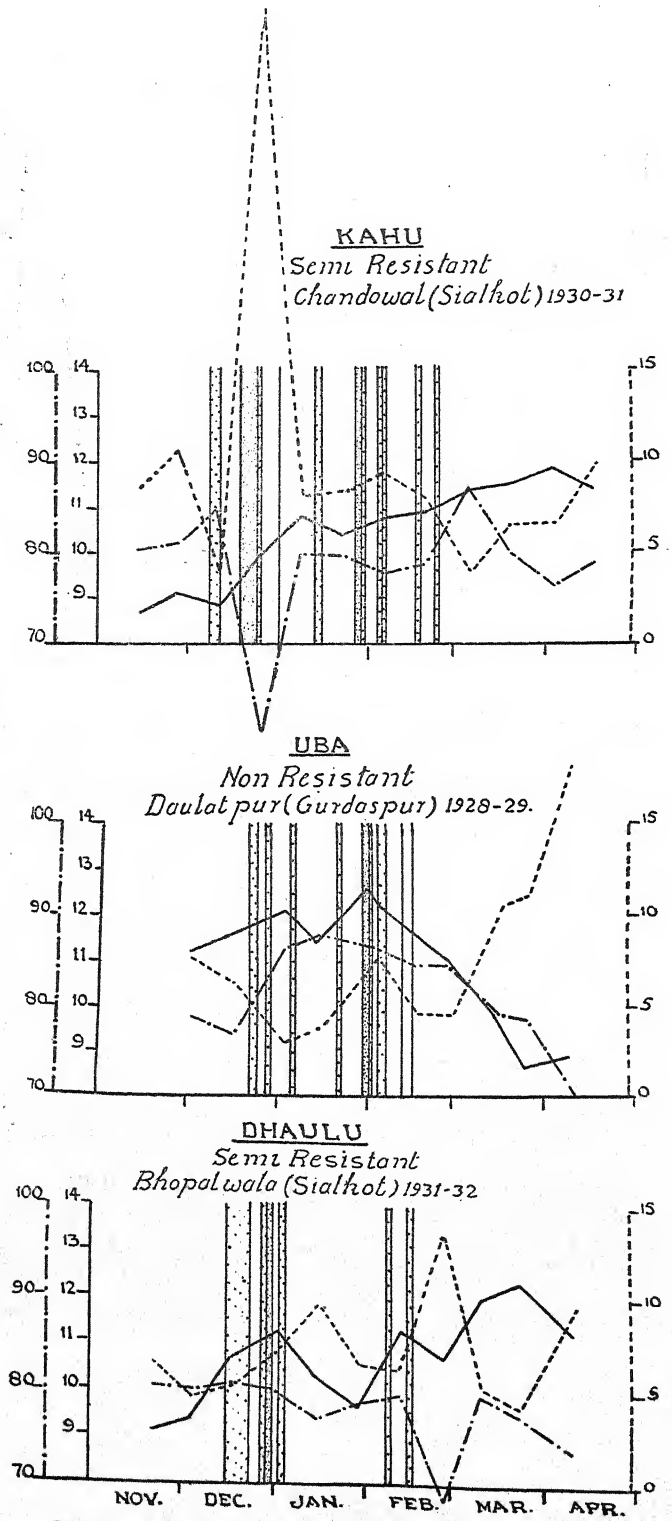


Fig. 7. Response to frost of three indigenous varieties

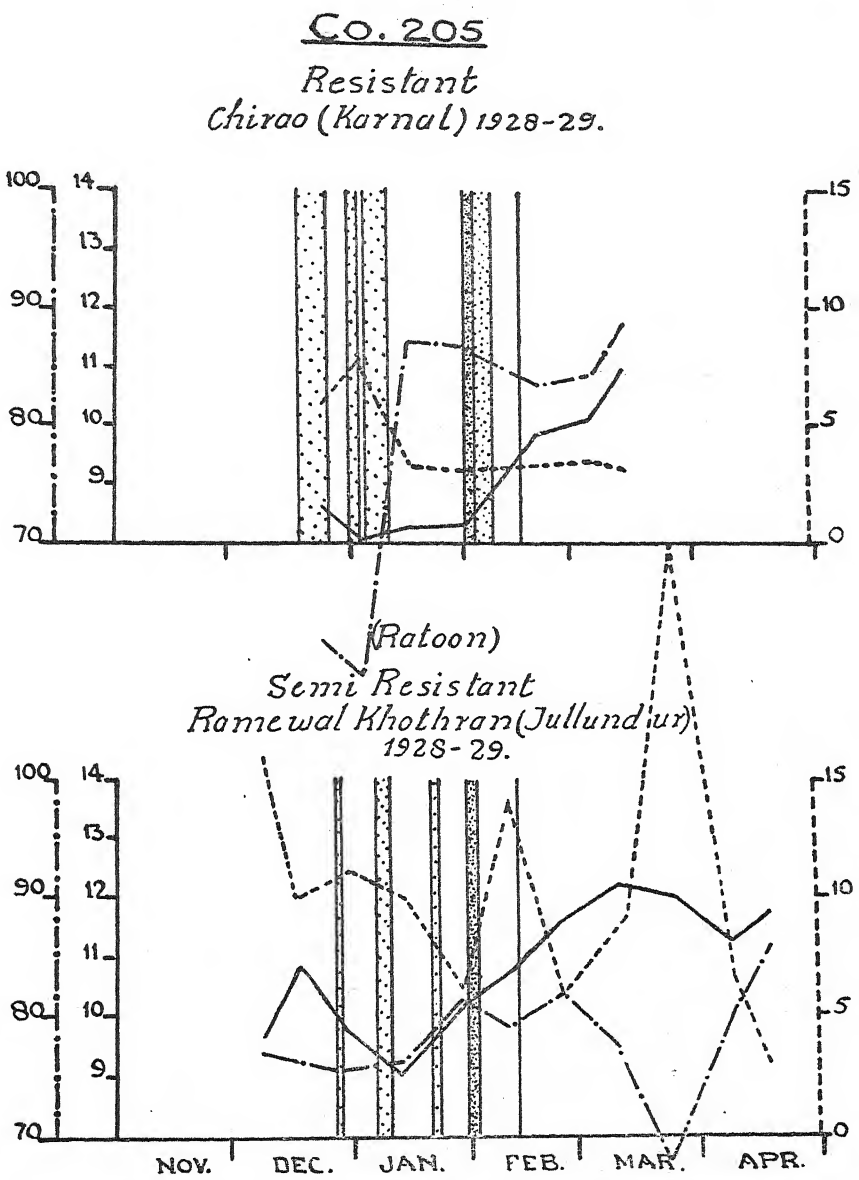


Fig. 8. Response of Co. 205 to frost as a resistant and semi-resistant cane

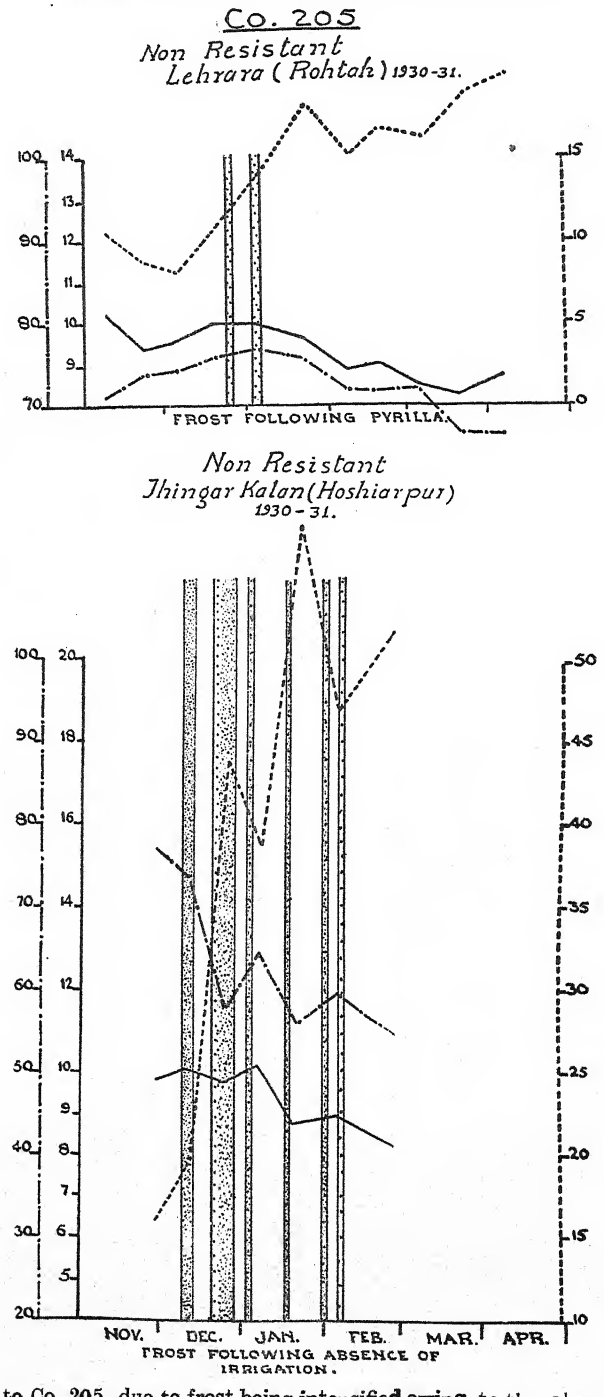


Fig. 9. Injury to Co. 205 due to frost being intensified owing to the abnormal condition of cane before frost

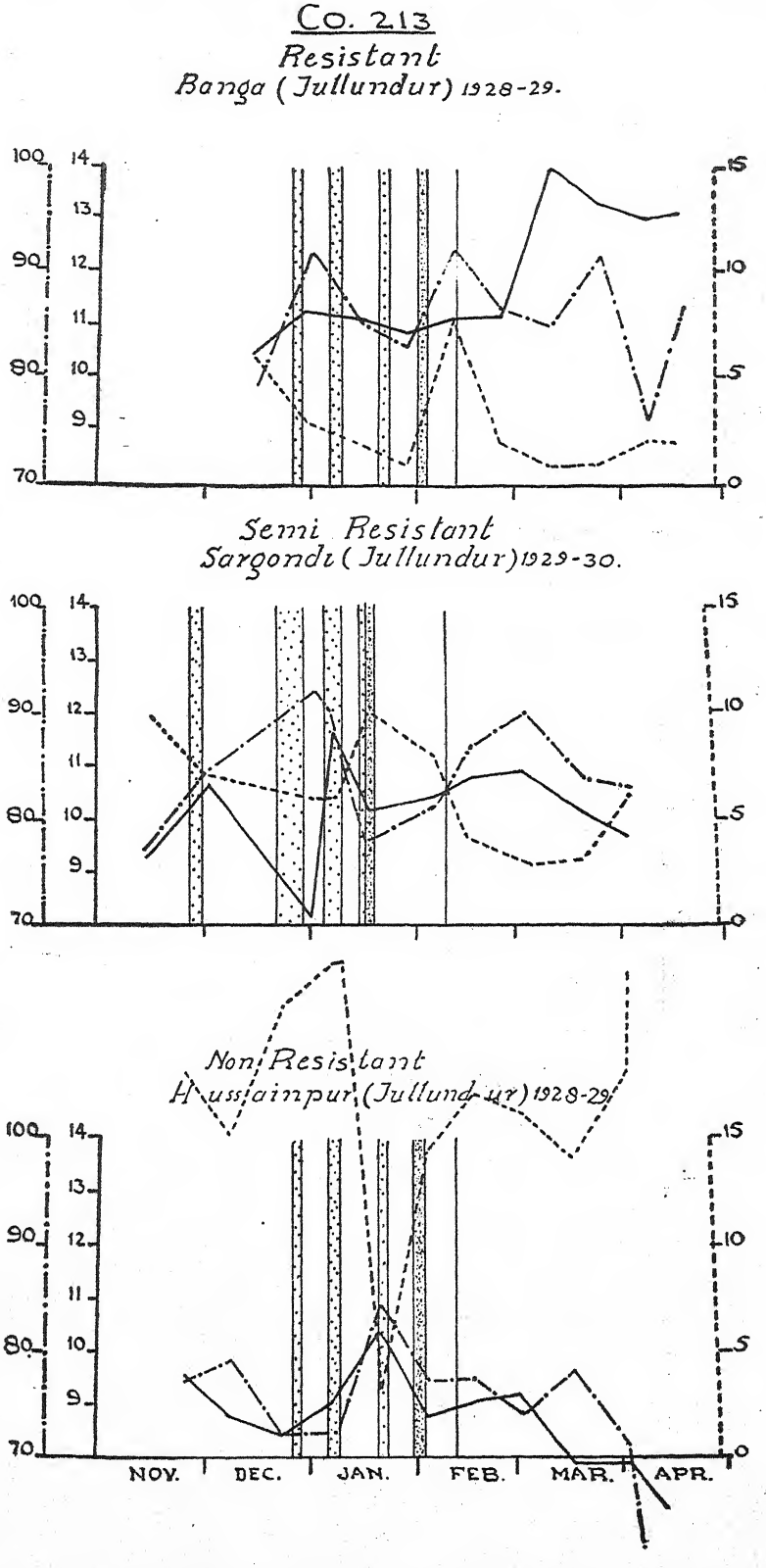


Fig. 10. Response of Co. 213 to frost as a resistant, semi and non-resistant cane

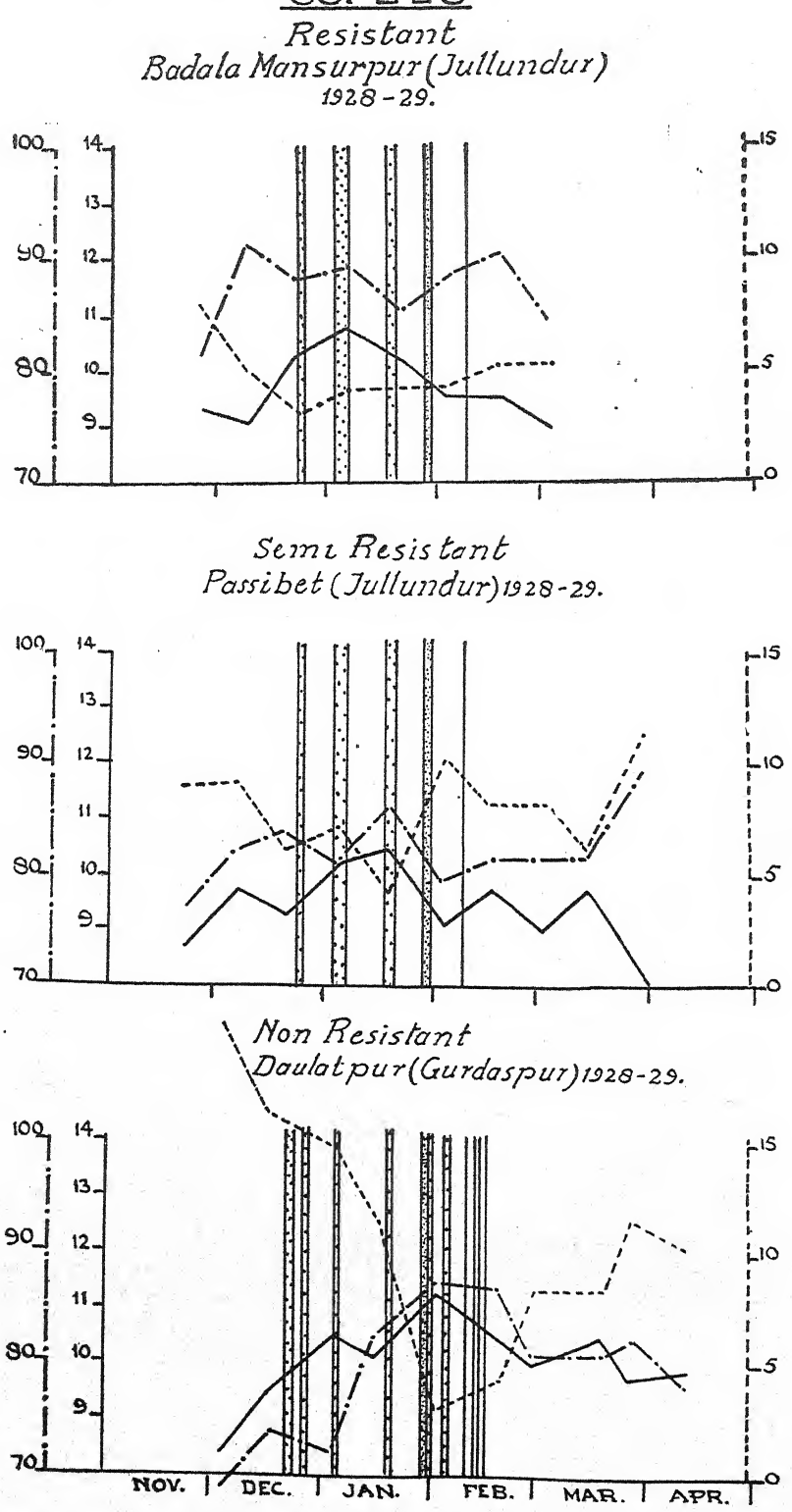


Fig. 11 Response of Co. 223 to frost as a resistant, semi and non-resistant cane

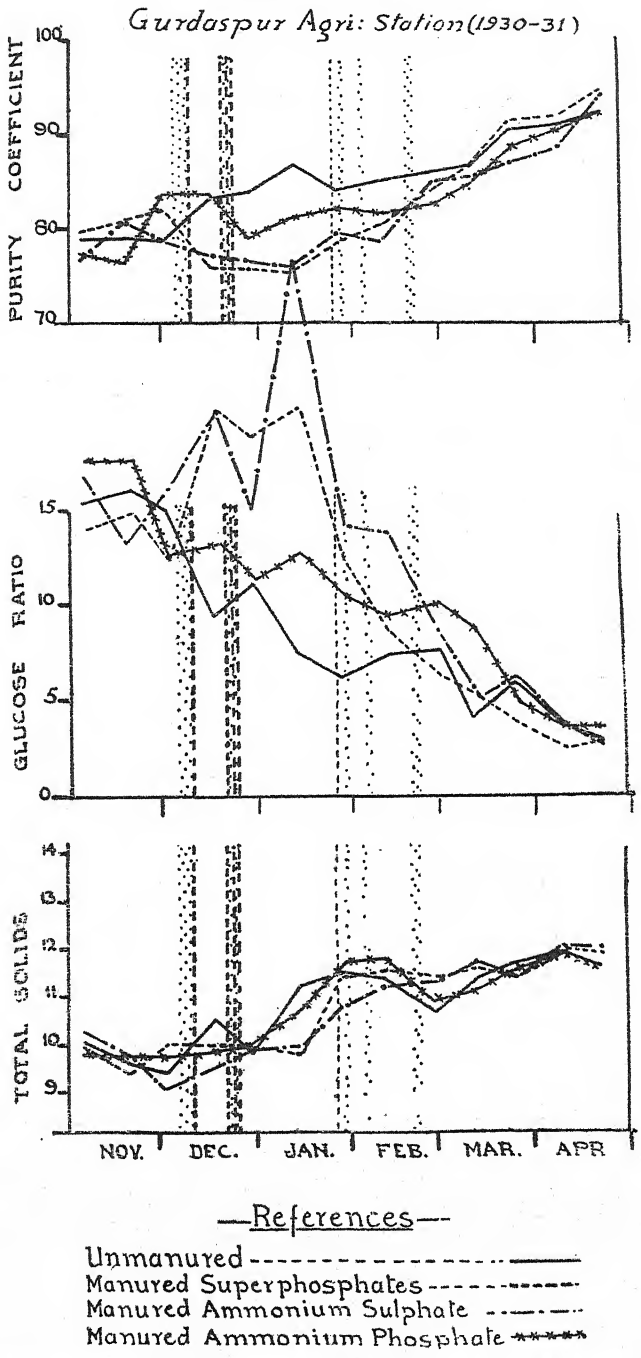


Fig. 12. Delayed maturity, without permanent injury, due to moderate cold following frost

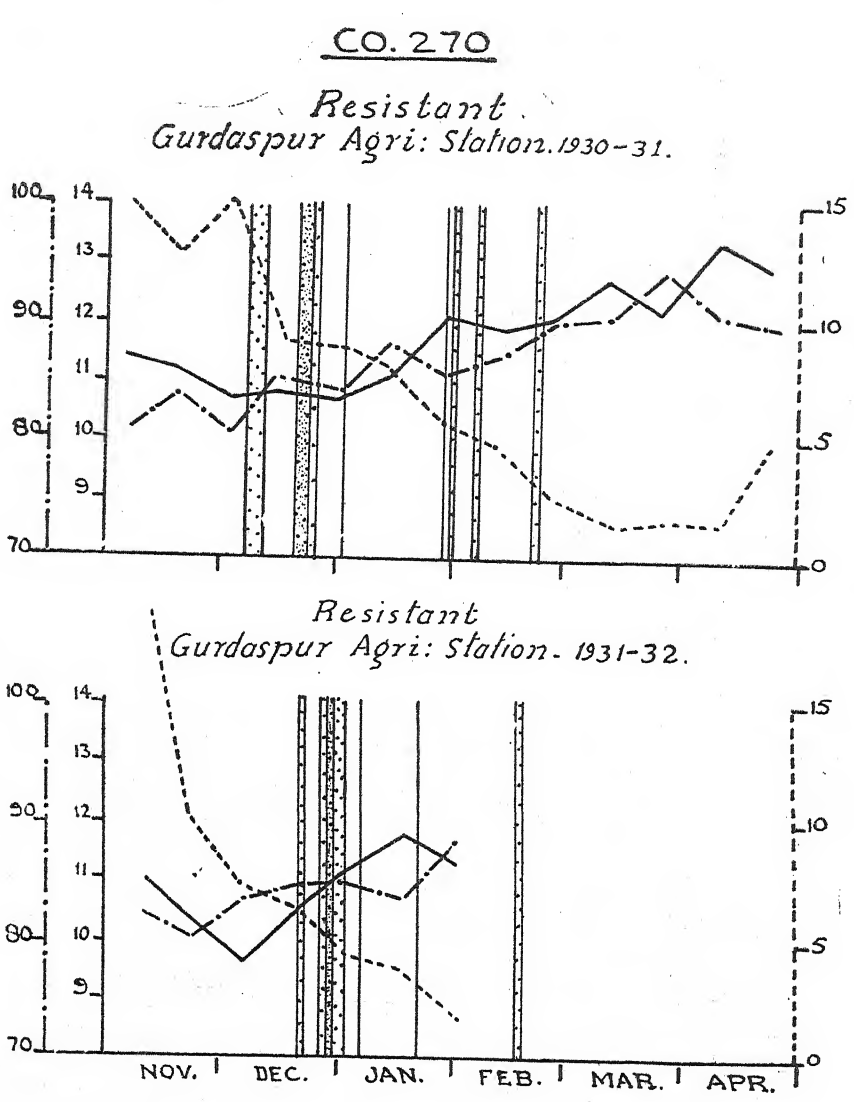


Fig. 13. Immunity of Co. 270 to frost

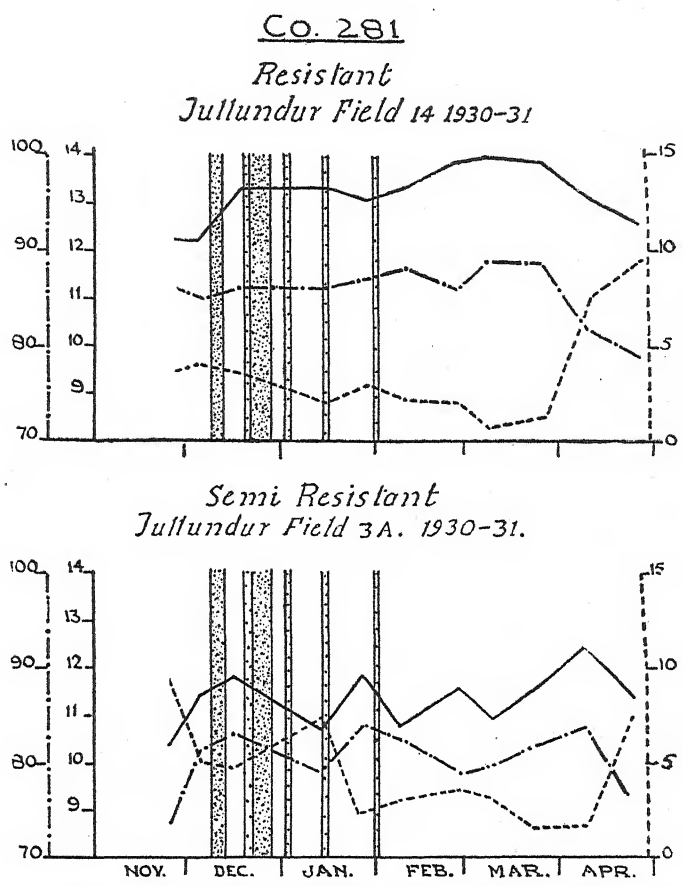


Fig. 14. Response of Co. 281 to frost as a resistant and semi-resistant cane

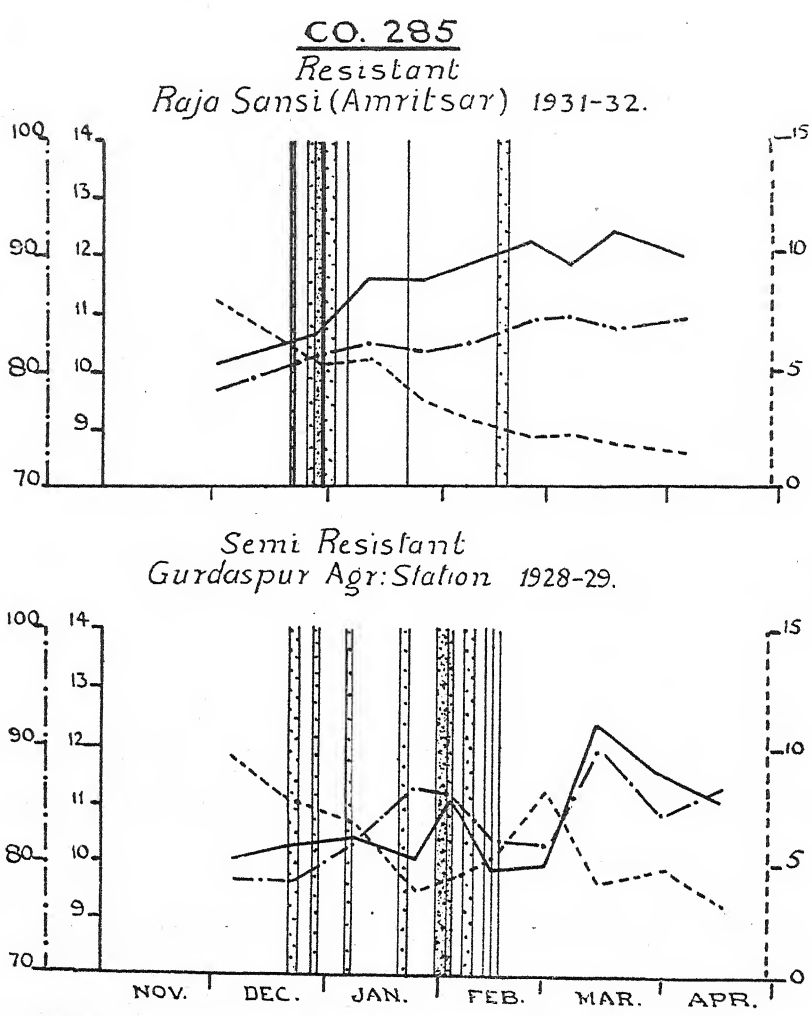


Fig. 15. Response of Co. 285 to frost as a resistant and semi-resistant cane

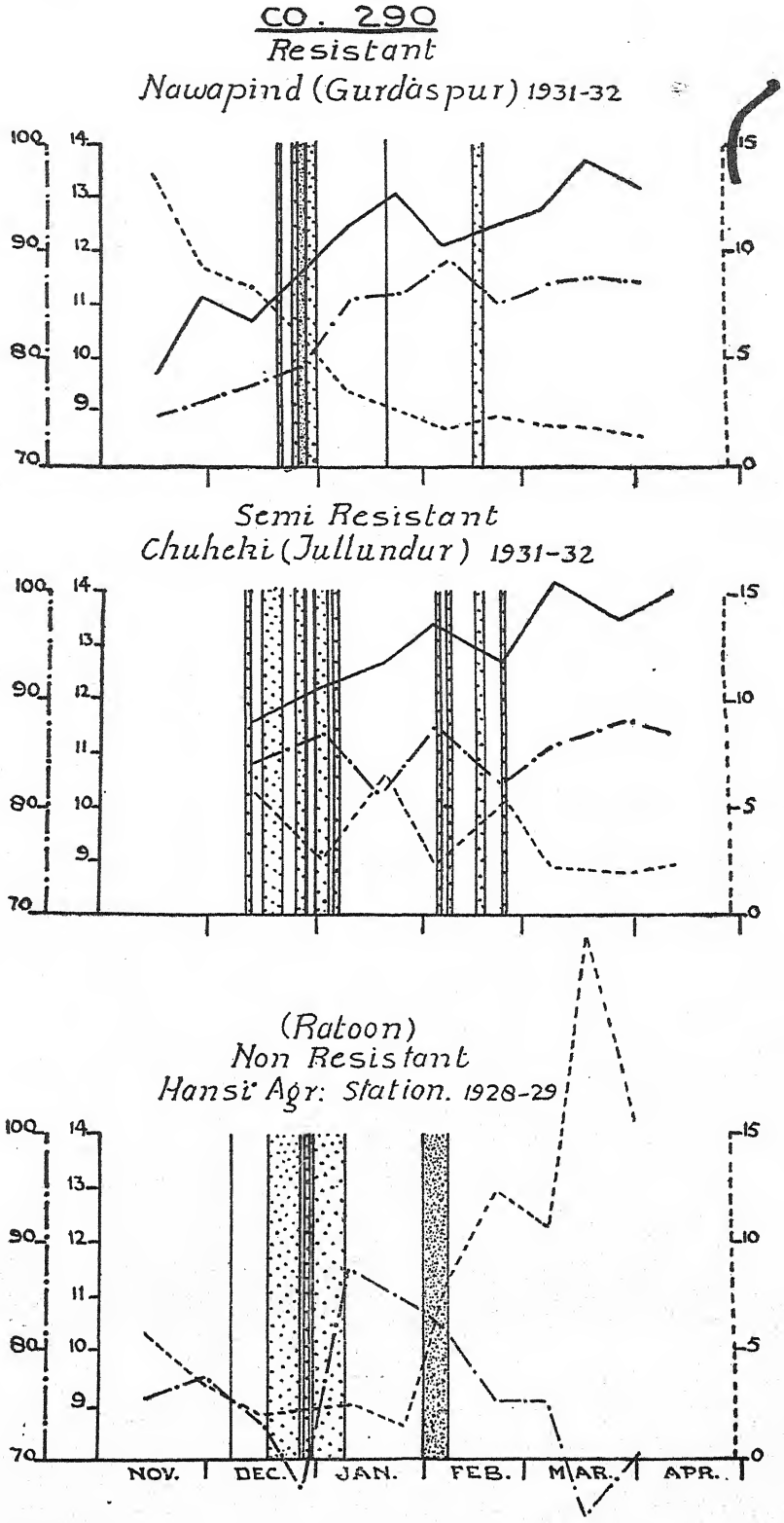


Fig. 16. Response of Co. 290 to frost as a resistant, semi and non-resistant cane

A STATISTICAL EXAMINATION OF THE YIELD OF WHEAT AT THE CAWNPORE AGRICULTURAL COLLEGE FARM, PART I*

BY

R. J. KALAMKAR, B.Sc., B.Ag., Ph.D. (Lond.),

Agricultural Meteorology Branch, Poona,

AND

SRIPAL SINGH, M.A., B.Sc.,

Agricultural College, Cawnpore

(Received for publication on 23rd March 1934)

(With two text figures)

The Cawnpore Agricultural College Farm is one of the earliest research farms started in India and may be expected to provide a useful series of data for statistical examination. The farm is situated in village Gotaiya, about three miles south west of the Cawnpore city. The land occupied was originally rented from the zamindars of Gotaiya in May 1881 and in that year the important manurial experiments whose data are under investigation were started. The soil of the farm may be accepted as a fair sample of the alluvial loam which occurs over a large portion of the Ganges-Jumna Doab.

The original records were brought to Poona for examination. From the vast amount of material a few fairly uniform series of experiments, *viz.*, the "*Kharif* standard series of maize" and the "*Rabi* standard series of wheat" were selected. These experiments had been carried on from 1885-86 to 1913-14 under more or less uniform cultural and manurial treatments. The present investigation deals with the analysis of yields of wheat of the "*Rabi* standard series" in which wheat (Muzzafernagar) was grown year after year. Eight out of the thirteen plots, each 400 square yards in size, are selected for the present investigation as they received a more uniform treatment year after year. The manurial treatments of the selected plots are as given below:—

Plot No. 1.—Saltpetre at the rate of 25 lbs. of nitrogen per acre.

Plot No. 2.—Saltpetre at the rate of 25 lbs. of nitrogen per acre + Bone dust at 10 lbs. of nitrogen per acre.

* The present investigation was made in the Agricultural Meteorology Branch (India Meteorological Department) financed by the Imperial Council of Agricultural Research. The data extends over a period of 29 years. The yields for the two years 1888-89 and 1890-91, were missing but for calculation the means of the yields of preceding and following years were taken for the study.

Plot No. 3.—Cow dung at 100 lbs. of nitrogen per acre.

Plot No. 6.—Sheep dung at 100 lbs. of nitrogen per acre.

Plot No. 7.—Ashes of cow dung containing 100 lbs. of nitrogen per acre.

Plot No. 9.—Saltpetre at 25 lbs. of nitrogen per acre + Bone super-phosphate at 10 lbs. of nitrogen per acre.

Plot No. 11.—Unmanured.

Plot No. 13.—Ashes of cow dung containing 100 lbs. of nitrogen per acre + saltpetre at 25 lbs. of nitrogen per acre.

Prior to 1899, however, the manures had been applied simply by weight without any reference to their actual composition [Leather, 1900]. Cattle dung and sheep dung were applied at the rate of 6·6 tons per acre, the nitrogen supplied by the former being about 75 lbs. and that by the latter about 145 lbs. Saltpetre of second rate quality was applied at the rate of 240 lbs. per acre which supplied on an average about 18 lbs. of nitrogen; 360 lbs. of bone dust containing from 9 to 12 lbs. of nitrogen were applied; and bone superphosphate containing on an average about 5 to 6 lbs. of nitrogen was given at the rate of 240 lbs. Since 1899 however the treatments are as noted against each plot.

The object of the present paper is to study the variation in yield from year to year on the above-mentioned eight plots, and to examine the relationship, if any between manurial treatments, mean yields and the variability of the yields. The influence of rainfall on wheat will be discussed in a later paper.

Method of analysis

The yield data for a series of years may be expressed as a polynomial function of time to examine whether there is any secular trend in the yield. The equation may then be represented thus:—

$$Y = a + bt + ct^2 + dt^3 + \ldots\ldots kt^r ,$$

where Y is the calculated yield and t represents the time. The values of yield calculated by this equation represent the general course of its changes due to factors which change in the sense as time progresses. The secular trend being thus allowed for, the deviations of the actual yields from the calculated may be ascribed primarily to the direct and indirect effects of the meteorological elements on the yield of the crop.

The above expression, however, is inconvenient as the values of the coefficients a, b, c, etc., change according as fewer or more terms of the series are used. Fisher [1921] has developed a polynomial method in which the coefficients obtained are independent of the number of terms employed.

The method developed by Fisher [1921] is used in this investigation. It consists in fitting polynomials to the various series of plot yields and analysing the total variation into the contribution due to (a) the average rate of deterioration of

soil due to the exhaustion of natural supplies of food ingredients or to the physical changes, (*b*) slow progressive changes which are of a complex nature and (*c*) annual variation which may be attributed to the direct and indirect effects of the weather elements on the yield of crops.

The fifth degree polynomial is fitted to the series of the wheat plots. The smooth curves are drawn to show the actual course and extent of the slow changes in the mean yield occurring in the differently treated plots. For the arithmetical Procedure Fisher's paper entitled " The influence of rainfall on the yield of wheat at Rothamsted " [Fisher, 1924] may be referred to. Precisely the same notation is used throughout the paper. The secular changes in the yields of differently treated plots are given in Table I. The measures x'_2 to x'_6 of the components of change of the 1st to the 5th degree are used since they are directly comparable with the standard residue.

Variability and its causes

As observed above, the variation in the yield is analysed into three parts (*a*) deterioration, (*b*) slow changes and (*c*) annual variation. The deterioration is represented by a linear function although theoretically it is probably more truly represented by an exponential curve, for, the rate of deterioration is expected to decrease as the capacity of the soil to produce as heavy a crop as previously is reduced, due to the depletion of the reserve supplies of plant nutrients in the soil.

Table I

Secular changes in the series examined

	Plot 1	Plot 2	Plot 3	Plot 6	Plot 7	Plot 9	Plot 11	Plot 13
x'_2 . .	—619·92	—133·99	216·53	215·11	—26·77	273·10	—311·75	—158·40
x'_3 . .	—1004·38	—1216·48	—1612·80	—1911·65	—1107·92	—2063·61	—1034·94	—1230·73
x'_4 . .	213·06	—370·63	36·19	215·39	1037·78	294·64	247·89	406·19
x'_5 . .	998·72	924·19	671·09	607·17	1274·86	951·84	866·90	479·30
x'_6 . .	53·92	—264·82	—346·27	—259·14	473·60	327·63	664·25	637·85
S. D. .	347·0	377·1	491.0	565·2	438·3	439·5	452·7	404·9

The total sum of squares of deviations in the yields for the 29 years with 28 degrees of freedom is split up into (*a*) sum of squares of deviations due to deterioration with one degree of freedom (*b*) sum of squares due to slow changes other than deterioration with 4 degrees of freedom and (*c*) the remaining sum of squares with 23 degrees of freedom due to annual causes.

The sum of squares with 23 degrees of freedom serves as a basis to test the significance of the deterioration or slow changes occurring in various plots. The results are given in Table II.

TABLE II

Analysis of variance

	Due to	D. F.	Mean square
Plot 1	Deterioration	1	384301
	Slow changes	4	513633
	Residual	23	120410
	Total	28	186009
Plot 2	Deterioration	1	17953
	Slow changes	4	635366
	Residual	23	142222
	Total	28	208233
Plot 3	Deterioration	1	46886
	Slow changes	4	792675
	Residual	23	241081
	Total	28	312945
Plot 6	Deterioration	1	46273
	Slow changes	4	1034155
	Residual	23	319380
	Total	28	411737
Plot 7	Deterioration	1	716
	Slow changes	4	1038514
	Residual	23	192072
	Total	28	306158
Plot 9	Deterioration	1	74587
	Slow changes	4	1339661
	Residual	23	193183
	Total	28	352730
Plot 11	Deterioration	1	97186
	Slow changes	4	581329
	Residual	23	204909
	Total	28	254836

TABLE II—*contd.*

	Due to	D. F.	Mean square
Plot 13 .	Deterioration	1	25092
	Slow changes	4	579068
	Residual	23	163920
	Total .	28	218269

Deterioration.—It will be observed from Table II that the mean square due to deterioration as represented by the linear term is on all plots smaller than the mean square due to the residual except on plot 1 which receives sodium nitrate. Even here the deterioration is insignificant. According to Fisher's table of 'z' [Fisher, 1931] for $n_1=1$ and $n_2=23$ the 5 per cent value of z is ·7269 which shows that the ratio of the mean square due to deterioration to the mean square due to residual should be of the order of 4·28, and here it is only 3·20 . The analysis therefore indicates that the deterioration as indicated by the linear term is insignificant on all the plots studied. It may be interesting to observe that the plots 3, 6 and 9, as indicated by the signs of x'_2 in Table I, show on the whole an upward tendency instead of diminution in the yield, although the effect is not significant in the light of the standard error.

Slow changes.—In Table I are given the values of x'_2, x'_3..x'_6, the five transformed co-ordinates with their standard error for all the plots studied. These co-ordinates give us the true estimates of the significant slow changes, if any, in the yields of wheat for the different plots studied. Slow changes in the series are exhibited by high positive or negative values in x'_2, x'_3, etc. The slow change due to deterioration as represented by the linear term x'_2 has already been considered. It is found to be insignificant on all the plots although on plot 1 it is comparatively high.

Slow changes other than deterioration are exhibited by x'_3, x'_4, x'_5 and x'_6 by their high positive or negative values. It will be observed from Table I that the values of x'_3 are negative and significantly higher for all the plots as judged in the light of their standard errors. For some plots some of the higher terms also are significant. Contribution to the total sum of squares made by x'_3, x'_4, x'_5 and x'_6 as due to the slow changes other than deterioration are given in Table II. According to Fisher's Table of 'z' [Fisher, 1931] for $n_1=4$ and $n_2=23$ the 5 and 1 per cent values of z are ·5140 and ·7251 respectively, indicating that the ratio of the mean square due to the slow changes to the mean square due to residual should be of the order of 2·8 and 4·3 for 5 and 1 per cent point respectively. It may be observed that the mean square due to the slow changes with 4 degrees of freedom

are significantly higher on all plots compared to the residual variance, particularly on those receiving sodium nitrate alone or in combination with phosphatic manure and also on the plots receiving ashes of cow dung alone.

The course and extent of the slow changes in the mean yield for the eight plots studied are given in Fig. 1. The proportionality of the slow changes is well brought out by plotting the yield figures on a logarithmic scale. All the curves in general show a very close similarity. There is a rise in the yield of wheat up to about 1900 after which period the yields decline. Some of the curves at their end points however show some dissimilarity. The rate of fall of yield is much slower on plots receiving sheep or cow dung, while the unmanured plot and plot receiving cow dung ashes show very rapid fall in yield. Other plots are intermediate.

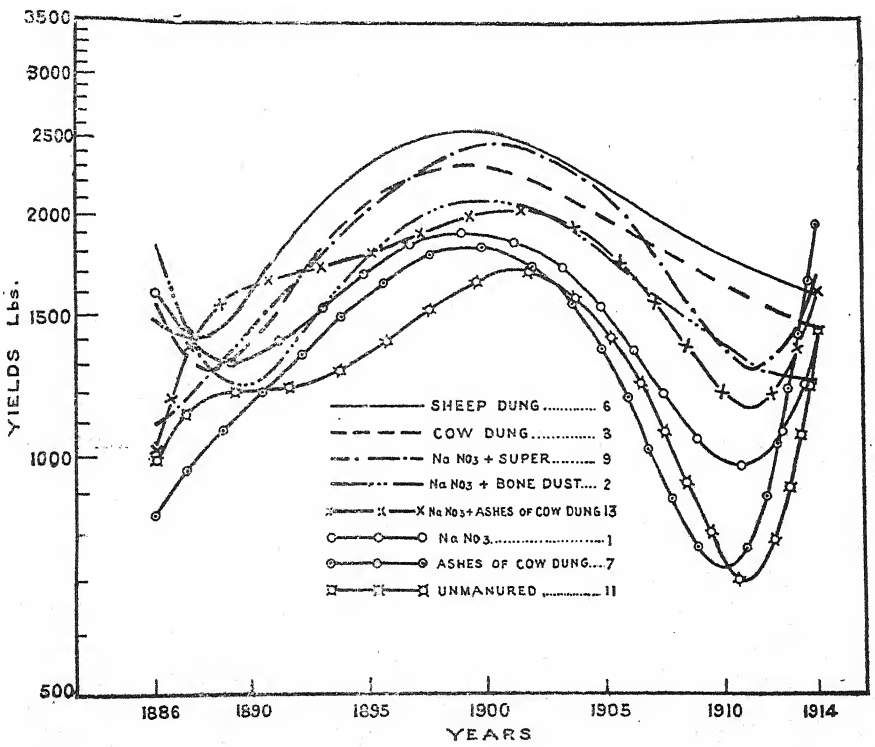


Fig. 1

It is difficult to assign authentic reasons to the slow changes that have taken place on all the plots. Records of the experiments do not contain any special notes such as changes in the type of cultivation,* radical changes in manuring both qualitatively and quantitatively, foulness of the plots due to weeds or surface erosion, etc., which are likely to throw some light on the causes of these changes.

* After submitting the paper one of the authors happened to read the remark made by Howard in his book "Wheat in India" that the marked increase in yield since 1895-96 is due to the deeper tillage and sub-soiling undertaken in that season than that formerly adopted.

Annual variation.—The annual variation for the differently treated plots are shown in Fig. 2. It is expressed on a relative basis. Deterioration and slow changes are also shown in this figure for ease and convenience in comparing them with the differently treated plots. The total sum of squares for each of the three types of variations is divided by the square of the mean yield for the respective plots and these figures are finally expressed as percentage. It may be observed from the diagram that the annual variation is most on plots Nos. 7 and 11, which were untreated for nitrogen.

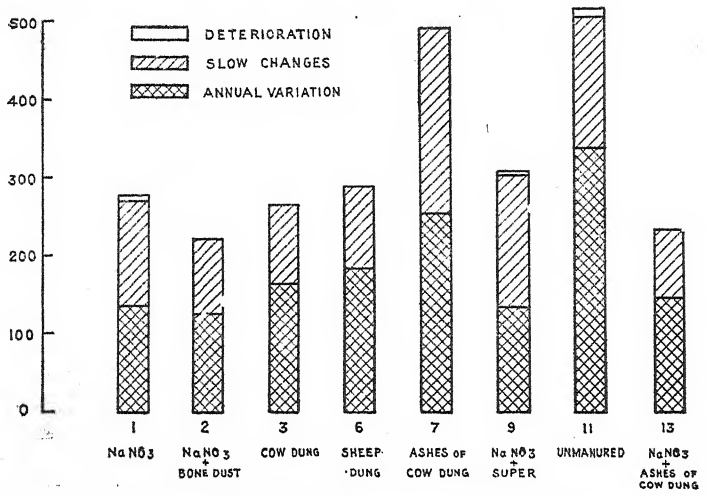


FIG. 2. CAUSES OF VARIABILITY IN THE YIELD OF WHEAT ON DIFFERENTLY TREATED PLOTS.

YIELD IN RELATIO TO MANURIAL TREATMENT

In the bulletin entitled "Experiments on the growth of wheat and maize at the Cawnpore Experimental Farm" [Leather, 1900], published in 1900, Dr. Leather has discussed data from 1883-84 to 1898-99. He has discussed in detail the effects of manures and fertilisers applied singly or in combinations and has compared the effects with the results obtained at Rothamsted and Woburn in England. The data now examined extend up to 1913-14 and confirm the conclusions arrived at by Dr. Leather in 1900 and further reveals the importance of the applications of phosphatic manures in combination with saltpetre which was not brought out by the earlier investigation.

Table III gives the mean yield of wheat with the standard deviation for each of the plots. The standard deviation is calculated from the sum of squares due to annual variation. The mean annual diminution or increment B, together with the standard deviation for the different plots is also attached. It may be indicated that they are obtained from the values of x'_2 given in Table I by multiplying by a constant, the relation being :

$$x'_2{}^2 = \frac{n\,(n^2-1)}{12}\,B^2,$$

where n is the number of years over which the data extend.

TABLE III

Plot	Mean yield	S. D.	Coefficient of variation	Mean annual decrement or increment, B	S. D. of B	Mean annual decrement or increment per cent	S. D. per cent.
1	1475·8	347·0	23·5	—13·76	7·70	—·93	·52
2	1631·1	377·1	23·1	—2·97	8·37	—·18	·51
3	1821·7	491·0	27·0	+4·81	10·90	+·26	·60
6	2005·7	565·2	28·2	+4·77	12·54	+·24	·63
7	1330·6	438·3	32·9	—0·59	9·73	—·05	·73
9	1805·3	439·5	24·3	+6·06	9·75	+·34	·54
11	1261·5	452·7	35·9	—6·92	10·05	—·55	·80
13	1634·3	404·9	24·8	—3·51	8·99	—·21	·55

The table shows that plot No. 6, receiving sheep dung, has given the highest yield. The sheep dung supplied contained on an average about 100 lbs. of nitrogen per acre. Plot No. 3, receiving cow dung containing an equivalent amount of nitrogen comes next. But an interesting point revealed by the table is that it is possible to obtain high yields by the aid of fertilisers containing no organic matter provided the fertiliser mixture is well balanced in respect of the food ingredients *e.g.*, plot 9 receiving saltpetre and super gave practically similar yield to plots 3 and 6 receiving sheep dung and cow dung respectively.

Another interesting point is that plot No. 1 receiving sodium nitrate alone does not give significantly higher yield than that of the unmanured plot No. 11. When however, sodium nitrate is applied in combination with phosphatic manures it

increases the yield. Superphosphate seems to be definitely better than bone dust. Plot No. 7 receiving ashes of cow dung gives slightly higher yield than the unmanured plot No. 11. Ashes of cow dung in combination with nitrogenous fertiliser increases the yield (plot No. 13).

We are grateful to Principal C. Maya Das, College of Agriculture, Cawnpore, for making the necessary data available and for the deputation of one of us to the Agricultural Meteorology Branch, India Meteorological Department, Poona.

The authors are also thankful to Dr. L. A. Ramdas, the Agricultural Meteorologist, for his help in the preparation of this paper.

REFERENCES

Leather, J. W. (1900). *Dept. Land Records and Agric., N. W. Province and Oudh, Bull. No.* 9.

Fisher, R. A. (1921). *J. Agric. Sci.* **11**, 107.

Fisher, R. A. (1924). *Philos. Trans.* **B213**, 89.

Fisher, R. A. (1931). "Statistical Methods for Research Workers" Oliver and Boyd, Edition 4.

SOME OBSERVATIONS ON THE SEED-SETTING IN A TYPE OF TOBACCO

BY

KASHI RAM,

Offg. Superintendent, Botanical Sub-Station, Karnal

(Received for publication on 29th October 1934)

(With plates XVI and XVII)

In 1929, while studying some of the pure lines of tobacco (*Nicotiana tabacum* L.) in the Botanical Section at Pusa it was observed that though Type 56 produced a large inflorescence with numerous flowers during December and January most of the flowers dropped without setting any seed.

Towards the close of the life of the plant, however, some capsules containing normal seeds were formed. In January 1930 the stigmas of certain flowers were observed to be surrounded with scanty pollen and on the next day when the flowers opened they became dark brown and the flowers dropped. Many of the anthers were dark brown when dehiscing and were observed to contain only a very small quantity of healthy pollen. When examined under the microscope, the pollen grains were found to be somewhat irregular in shape but some of them were healthy and sent out pollen tubes when germinated in 3 per cent sugar solution.

In order to obtain definite information about this peculiar behaviour of Type 56, it was sown along with Type 63 as control, late in August and transplanted early in October 1930. A number of flowers opening at different times were marked and the number that formed capsules was noted.

The results are set out in Table I.

TABLE I

Interval of time	Type 56			Type 63		
	No. of flowers marked	No. of capsules formed	Percentage setting	No. of flowers marked	No. of capsules formed	Percentage setting
22nd Dec. 1930 to 23rd Jan. 1931	96	34	35·4	106	101	95·3
24th Jan. 1931 to 12th Feb. 1931	80	42	52·5	64	62	96·9
13th Feb. 1931 to 28th Feb. 1931	96	85	88·5	50	49	98·0
1st March 1931 to 14th March 1931	75	64	85·3	35	33	94·3

From Table I it is clear that there was much flower shedding in Type 56 during December and January (Plate XVI), but with the lengthening of day light and the relative rise in temperature during February and March there was fairly good setting (Plate XVII).

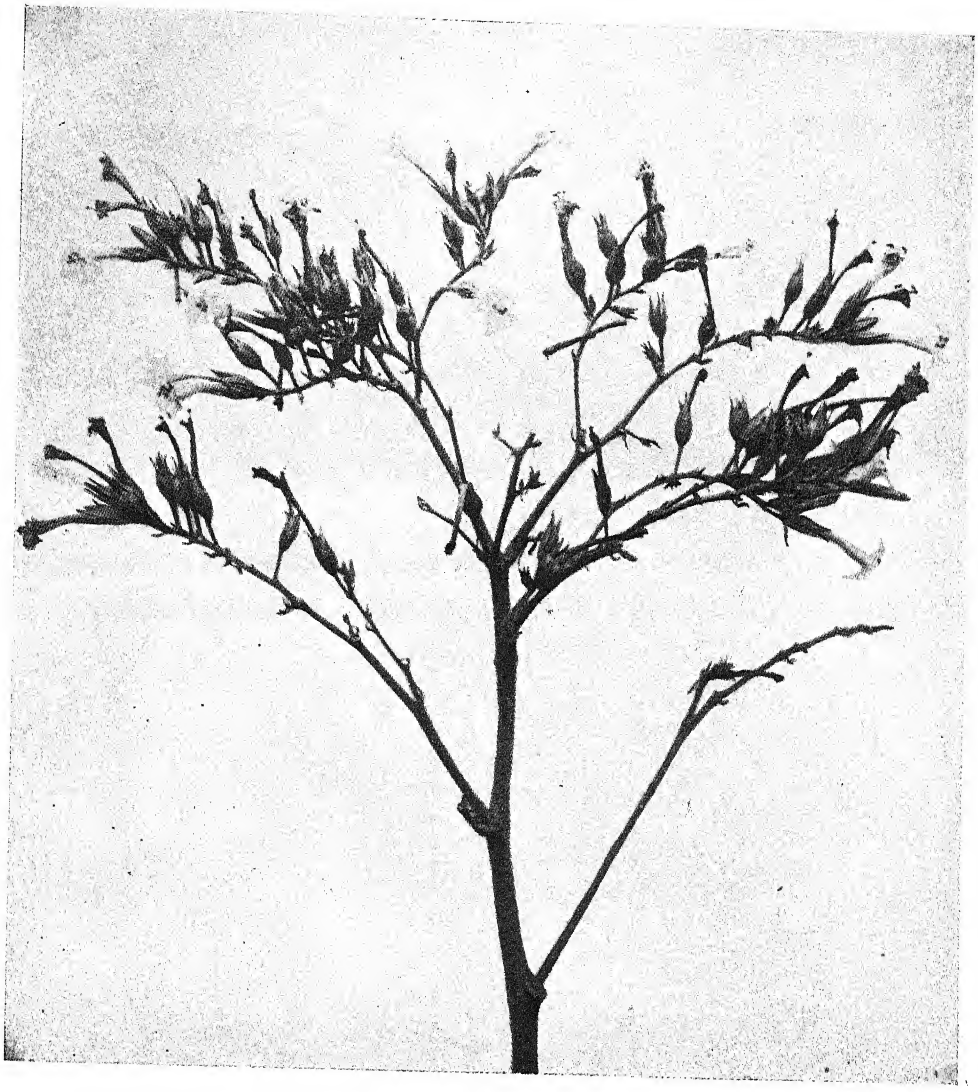
In order to know whether the ovary was receptive during December and January a number of flowers of Type 56 were pollinated with pollen from Type 63 which does not show any lack of setting in the cold season. A good set of seed was obtained as the following figures show :—

Cross	Date of pollination	No. of flowers pollinated	No. of capsules set	Percentage setting
T. 56 T. 63 . .	1st Dec. 1930 .	9	8	89
T. 56×T. 63 . .	28th Jan. 1931 .	8	6	75

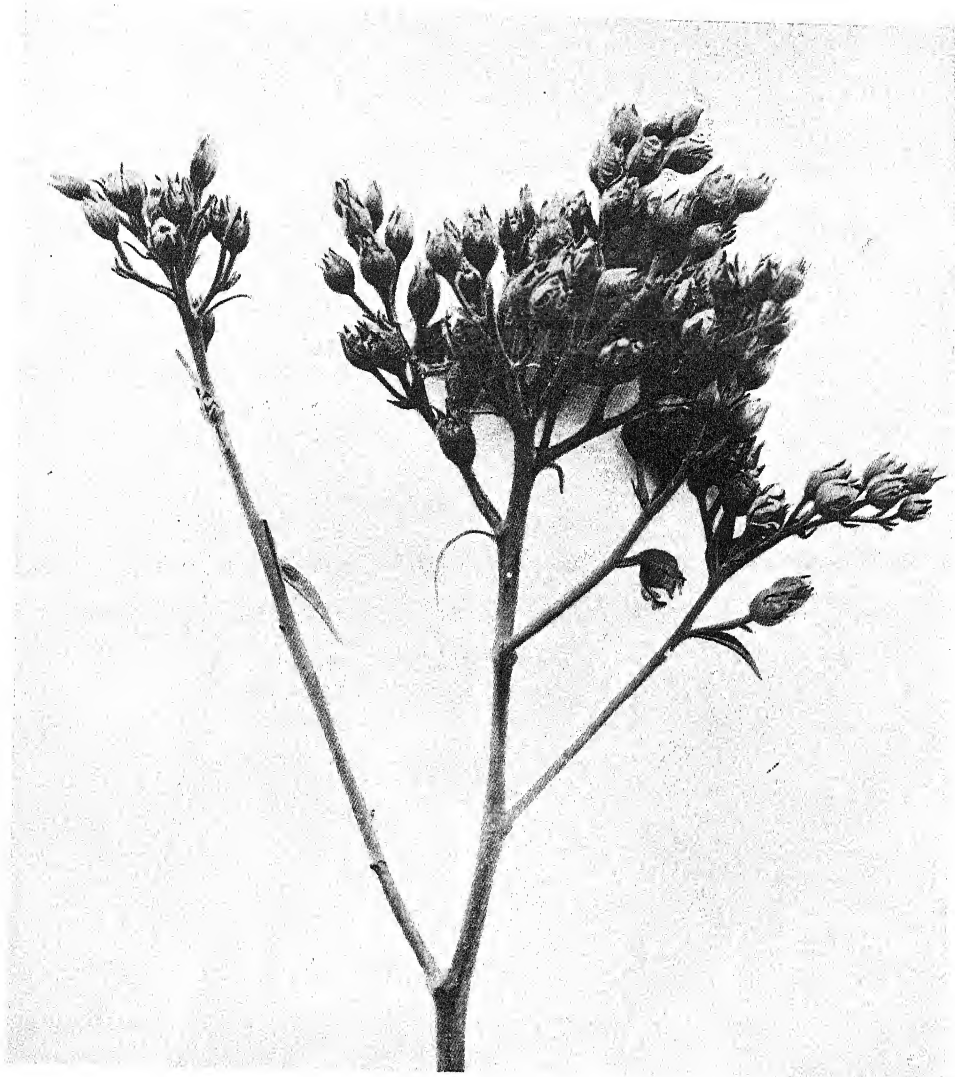
This suggests that there is some defect in the pollen of Type 56 during cold season with short day light. To make a positive test of this, crosses were made using Type 63 as the female parent and Type 56 as the male parent with the results shown below :—

Cross	Date of pollination	No. of flowers pollinated	No. of capsules set	Percentage setting
T. 63×T. 56 . .	1st Dec. 1930 .	12	7	48
T. 63×T. 56 . .	28th Jan. 1931 .	7	3	43

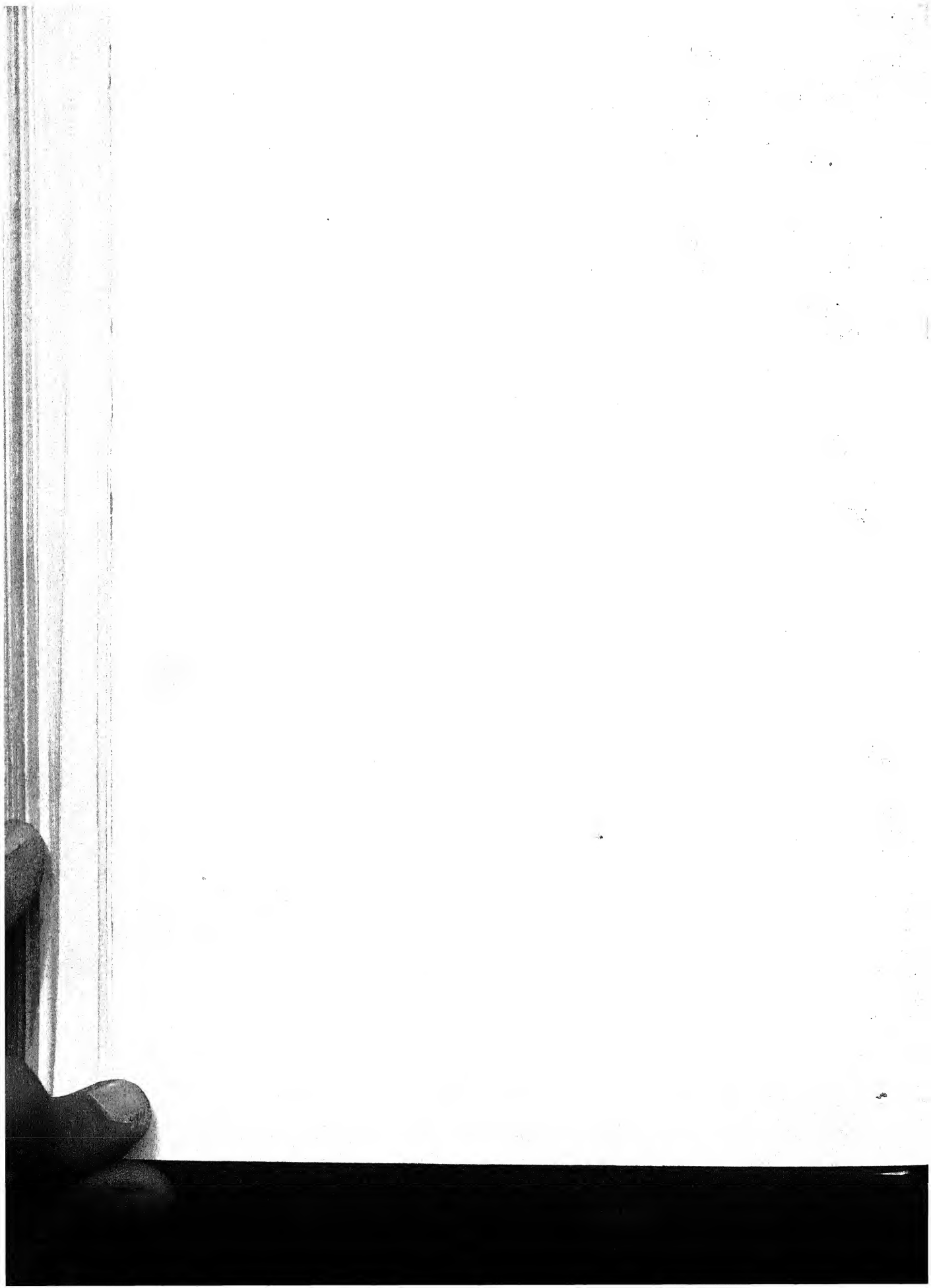
From the figures given above it is clear that the pollen of Type 56 did not function properly, giving a lower percentage of setting than in the reciprocal cross. In order to confirm this observation the experiment was repeated again in 1933 on a more extensive scale. Type 56 along with the control Type 63 was grown and transplanted at different times of the season. A large number of flowers in both the types was marked and selfed. The development of a pinkish tinge on the flower bud is generally an indication that the flower will open next morning; hence in selfing care was taken to gum all such flower buds by applying ordinary rubber solution to their apices, the evening previous to the actual opening of the buds.



Type 56, flowering from 22nd December 1930 to 23rd January 1931
(Poor setting of seeds).



Type 56, flowering from 1st March 1931 to 14th March 1931
(Good setting of seeds).



The number of flowers thus marked or selfed and the number of capsules formed were recorded and these are given in Table II.

TABLE II

Period	Mean maximum temperature	Mean minimum temperature	Type 56			Type 63		
			Number of flowers marked	Number of capsules formed	Percentage setting	Number of flowers marked	Number of capsules formed	Percentage setting
A.* 24th Jan. 1934 to 12th Feb. 1934	72·6	48·0	542	86	15·9	141	135	95·7
13th Feb. 1934 to 28th Feb. 1934	78·3	58 3	1713	682	39·8	495	457	92·3
1st March 1934 to 14th March 1934	84·6	57·9	1509	1152	76·3	533	493	92·5
15th March 1934 to 2nd April 1934.	92·7	61·5	Plants damaged later in the season.					
B. 24th Jan. 1934 to 12th Feb. 1934	72·6	48·0	69	12	17·4	29	27	93·1
13th Feb. 1934 to 28th Feb. 1934	78·3	58·3	843	289	34·3	427	397	92·9
1st March 1934 to 14th March 1934	84·6	57·9	1128	926	82·1	719	665	92·5
15th March 1934 to 2nd April 1934	92·7	61·5	1641	1469	89·5	591	565	95·6
C. 13th Feb. 1934 to 28th Feb. 1934	78·3	58.3	437	160	36·6	12	11	91·7
1st March 1934 to 14th March 1934	84·6	57·9	836	697	83·4	226	204	90·3
15th March 1934 to 2nd April 1934	92·7	61·5	951	872	91·7	437	420	96·1
D. 13th Feb. 1934 to 28th Feb. 1934	78·3	58·3	66	23	34·8	3	3	100·0
1st March 1934 to 14th March 1934	84·6	57·9	599	491	82·0	192	178	92·7
15th March 1934 to 2nd April 1934	92·7	61·5	1032	910	88·2	468	435	92·9

A reference to Table II brings out the fact that in the reproductive activity of Type 56 there is some cause which interferes with the normal functioning of the pollen in fertilizing the ovary during short duration of day light and at low temperatures prevailing in December and January so that seed formation is greatly limited.

SUMMARY

In Pusa Type 56 tobacco few capsules are formed during the cold weather with short day light prevailing in December and January but the number increases as the day light brightens and the temperature rises in the succeeding months.

Observations on reciprocal crosses between this type and Type 63 which shows good setting throughout the season indicate that the cause lies in the defective functioning of the pollen of Type 56 during short duration of day light and at low temperatures.

* The seeds were sown in four lots, designated A, B, C and D, at intervals of about a fortnight.

ABSTRACT

Agricultural Turkey. 581. 9 (47). 63 (47). P. ZHUKOVSKY. (*Lenin. Acad. Agric. Sci., Inst. Pl. Ind. Moscow and Leningrad* 1933 Pp. xxvii+908; 381 figs., 12 pls.) (Full summary prepared by the Imperial Bureau of Plant Genetics, Cambridge)

This large tome, comprising some 800 pages of original text represents the fruits of three Soviet expeditions, headed by the author, to Asia Minor. The expeditions have been the means of accumulating a vast amount of plant material, much of it new a d of extreme value, some for immediate introduction, some for breeding.

The general characteristics of the country and its climate are described, followed by chapters on the natural and agricultural flora of the principal zones and the agriculture of the region in general.

In the second section, comprising the main body of the work, detailed descriptions are given of the forms and varieties collected in each individual crop, constituting an invaluable contribution to the Soviet fund of initial breeding material. Particular interest is attached to the discovery of spring forms of *Triticum durum*, among which appear some extremely early varieties, others tolerant of high temperatures, *Fusarium* and frit-fly; barleys have been found with unusually large grain and high yielding capacity and with high protein content; poppies with record earliness, opium content and drought resistance; greatly superior forms of aniseed; melons excelling any in the world in sweetness, juiciness, thickness of the flesh and transportability; cold. resistant winter oats, *Avena byzantina*; sesame resistant to *Fusarium*; flax capable of yielding both fibre and oil; *Phaseolus* immune to anthracnose; and a great abundance of forms of rye and a number of other crops, in some character or other superior to the ones previously known.

The investigations have succeeded in demonstrating that Anatolia is the centre of origin of many of the best European cultivated plants. The cultivated type of soft wheat, the squarehead type in particular, apparently originated in Anatolia and penetrated into Europe later; the same is true of the cultivated forms of the European leguminous plants, the best forms of sesame, the table carrot, cultivated aniseed, the cantaloupe and casaba melons, cucumbers, French lucerne, the opium poppy, various fruit trees and wine grapes. Literally thousands of cultivated forms of these several plants have been found. In wheat the greatest variety is found in *T. durum*, including many endemic forms and characters. The well-known Kubanka wheat is present in great variety, indicating that it too has in all probability entered Russia from Anatolia. There are endemic forms and characters also of *T. turgidum* and two species of wild wheat are present in abundance. The country is divided into very varied climatic regions and in consequence the wheats display a wider range of ecological types than those of any other *durum* wheat country; even in Abyssinia, the country of the greatest

botanical diversity, the ecological type is relatively uniform. Some of the new forms of *T. durum* have already been used in crossing on account of their low temperature requirements, tolerance of excessive moisture and immunity to *Fusarium*, all of which characters are required for extending the northern limit of *T. durum* cultivation. The variety *asiaticum*, besides being immune to *Fusarium*, entirely escapes frit-fly attack owing to its earliness, low tillering and vigorous and rapid early growth. The dwarf *T. durum* comes into ear earlier than any other known forms and is hence extremely valuable for certain parts of the U. S. S. R. Hybrids of this are in the third generation. Another valuable breeding character of these wheats is the high quality of their grain, the variety *horanicum* having excellent vitreous grain of spherical shape, maturing very early. Some of them also respond favourably to vernalization.

The botanical variation in the wheats is equally great, especially in the Mediterranean region of Anatolia, which is regarded therefore as an important, though not the primary, centre of origin of the 28-chromosome wheats. The 42-chromosome group shews much less diversity, except *T. compactum*, which may have a secondary centre in Anatolia and Transcaucasia. A new and endemic 42-chromosome species, *T. vavilovi* characterized by branched ears, has been discovered, however, and is here described. The 14-chromosome group of wheats and the genus *Aegilops* have their centre of greatest diversity in Anatolia and the Crimea and two new species of *Aegilops* have been found there.

The barleys of Anatolia are much less varied but nevertheless are of breeding value or their drought resistance, strong straw, non-shattering ears and large elliptical grain which shews considerable variation in protein and starch content.

Rye is found universally, as a weed in all the wheat fields and also as a pure crop. These ryes display a greater diversity of varieties, forms and characters than is found in any other country, in the form and colour of the ear which varies from yellow to black, in the form and colour of the grain, varying from elongated to nearly spherical and from yellow, green and purple to nearly black ; awnless forms appear, forms with smooth, pubescent and warty glumes ; forms with branched ears, others with three grains per spikelet, types with wax and types without, and certain self-pollinating ryes. Many of these forms are new and endemic ; a new wild species, *Secale ancestrale*, has been found in great abundance and in a great variety of forms, as well as the commont wild *S. montanum* and the allied genus *Haynaldia villosa* (*Secale villosum* L. syh. *Triticum villosum*). The extreme heterozygosity of the ryes of this region is thought therefore to be the result of frequent intercrossing between the various forms and species and there is no doubt that this is the centre of origin. *Secale fragile* also occurs but there is evidence that the area of development of the hybrid forms is distinct from that of the original species. *Haynaldia hordeacea* is thought to have possibilities for crossing for producing a perennial rye for the north and for arid zones ; similarly *H. villosa*, *S. ancestrale* and *S. montanum* for producing drought-resistant forms and forms suitable for sandy districts.

Avena byzantina has been grown in Anatolia from ancient times. It is cultivated as a winter crop and is a common weed. It is present in a great variety of forms, some endemic ; it is rich in winter and semi-winter forms which are of great use in certain parts of the U. S. S. R.; and in addition to cold resistance they have large grains with high

content of protein and oil, and are resistant to rust, shattering and lodging ; some are also very early and altogether this is regarded as the most valuable material for oat breeding yet found. There is a great variety also of wild *Avena* and Anatolia is regarded as the centre of origin of a whole group of oat species ; the process of its gradual conversion from a weed to a cultivated plant can be observed at the present time in the wheat fields ; the weed forms contain a much greater wealth of variation than the cultivated forms and many of them are endemic. Natural hybridization is common and the progeny of these natural hybrids often contains further new forms, some of which moreover are extremely similar to Burt and other new hybrids obtained from the U. S. A.

Maize and rice, which are introduced crops, are much more uniform.

Many of the leguminous plants on the other hand display a tremendous variety, *e.g.*, the variation in seed form, vegetative period and morphological characters in the grams (*Cicer arietinum*) mark it as one of the most valuable known sources of breeding material. Anatolia is second only to Persia in richness of species of *Cicer* ; there are five wild and two endemic species, though the supposed wild ancestor of the cultivated gram *C. judaicum* is not present. The examination of the wild species leads to the suggestion that several of them have contributed to the origin of the cultivated forms, possibly partly by hybridization ; this view can only be substantiated by genetic study of the wild species. Anatolia contains a large number of endemic varieties recessive characters being predominant ; the Anatolian grams excel all others in quality and most others also in yield.

Of lentils a considerable range of varieties is found, including the intermediate as well as large and small-seeded varieties of *Lens esculentum* and all the four wild species. In opposition to Barulina's view the author thinks it likely that the large-seeded forms have arisen by hybridization of the original small-seeded form with some of the wild species such as *L. Kotschyana* or *L. orientalis*, leading to the independent origin of lentil cultivation in the Mediterranean, where the different type of cultivation, *e.g.*, winter sowing, made possible the evolution of the forms with larger seeds. The position with regard to beans (*Vicia Faba*) is different, in that the small-seeded forms are much rarer, no endemics are found and the general character of the crop corresponds to that of the Mediterranean countries. They are nevertheless of great practical interest for breeders on account of their large beans, earliness and high quality ; similarly with regard to *Phaseolus*, some of which are resistant to drought and others to anthracnose.

Although Anatolia is the home of all the wild species of *Pisum* hitherto known, *P. sativum* is very little cultivated and the forms cultivated are much less varied than those of Afghanistan and Abyssinia. Two new species have recently been discovered in Georgia and these may shed light on the origin of the cultivated pea ; at present however there is very little relationship between the cultivated and the wild species. The Anatolian group represents a distinct type from the peas of Afghanistan or Abyssinia ; in ecological characters it is very much more varied, including the reaction to reduced length of day, and although there are many isolated characters shewing the influence of Afghanistan, Transcaucasia and the Mediterranean countries it is clear that the peas of Anatolia have for a long time evolved independently.

Vetches on the other hand are present in remarkable abundance and variety, both as wild species growing as weeds and cultivated forms of *Vicia sativa*, the majority of which are endemic. Most of the characters are common to the cultivated and weed forms, the latter being richer in characters and it seems probable that in this region the process of "domestication" is still in progress. The group is in every way distinct from the two sub-species hitherto recognized and is established as a separate sub-species, *turcica*. Among the general characteristics of this group are smallness of plant, extreme earliness, cold resistance, abundant seed production and firm attachment of the seed coat. Earliness and abundant seed production are characteristic of the Anatolian *Lathyrus*, of which two cultivated species *L. sativus* and *L. ochrus* occur.

Special interest is attached to the sesames on account of their great diversity and their high quality. Most of them have white seeds, these varieties being endemic; they are also characterized by high oil content and very pronounced resistance to diseases such as *Fusarium*, bacteria and gumming. Of linseed too there is a whole endemic group, intermediate in character between the Asiatic and Mediterranean groups; there is a further prostrate form in many ways resembling the wild forms, endemic for Anatolia and Transcaucasia, and capable of withstanding very low temperatures and growing in poor, sandy situations. The high yield of capsules and the large seeds, combined with their high oil content, have directed the attention of breeders to the Anatolian linseeds.

Anatolia is apparently the home of aniseed. *Pimpinella anisum* grows wild in certain parts of the country and there is an unusual variety of cultivated forms. These yield more heavily than the forms of any other country, though in essential oil content they are inferior to the Spanish forms. However, a new species, *P. anisetum*, endemic in eastern Anatolia, has been found remarkable for its high oil content (8·3 per cent as compared with 3·4 per cent for *P. anisum*), its vigorous growth, high yield and pronounced cold resistance. It is a biennial and very resistant also to fungous diseases. Its one defect is that the essential oil it yields is of a somewhat lower quality, containing as it does a smaller proportion of anetol.

Wild growths of oleander, jasmine, rosemary, lavender (*Lavandula Stoechas* L.) and other aromatic plants are also found in abundance.

The local cotton is *Gossypium herbaceum* but adventive species such as *G. hirsutum* and *G. punctatum* are also present, all of distinctly low quality.

A special endemic sub-species of the opium poppy, consisting of several distinct ecotypes, is described. It is regularly accompanied by semi-cultivated types belonging to the dehiscent sub-species, *semispontaneum*, thought to be nearly allied to the wild ancestor of the cultivated poppy. The high morphine percentage and early maturity of the cultivated forms, together with their tolerance of both cold and drought have attracted the attention of Soviet breeders. Their yield of opium is unfortunately low and they are being crossed with Soviet forms having high opium content in the attempt to combine this with their high morphine percentage which in some cases was as high as 28·33 per cent.

A study of the present distribution of the cultivated poppy together with the genetic and cytological investigation of it and of the Mediterranean wild species *Papaver setigerum* have led to the conclusion that this is not the wild ancestor of *P. somniferum*.

The greatest number of forms and characters is found in the Central Asiatic Republics of the Soviet Union, where three endemic sub-species occur and which must therefore be regarded as the scene of origin of the cultivated species. In Turkey also there occur primitive forms with dehiscent capsules, and with warty capsules of the type used for producing edible seeds and the present Anatolian and Persian forms are thought to have evolved later from forms originally coming from Turkestan. The great variety and wide distribution of cultivated poppies of the present day have arisen monophyletically, as the result of man's agency.

Each vilayet has its own peculiar type of melon, resulting from age-long selection independently of neighbouring vilayets. An untold wealth of forms has thus originated. Of these frequently only the most inferior have penetrated into other countries. The true melons constitute an endemic group *gracilior*, some of which are of exceptionally high quality, with a sugar content higher than any known melon, exceeding 9 per cent in some cases. One of the casaba melons found has been pronounced the best melon in the world on account of its combination of sweetness, juiciness, thick flesh and "buttery" consistency. It is to be used in crossing with more aromatic types of melon. The vilayet of Van is famed as the home of the cantaloupes and still contains a great variety of forms, many of which have not hitherto penetrated into Europe, whilst the snake melons also have their centre of diversity in Anatolia. Of water-melons both the cultivated and weed forms exist in great diversity, also as a special group *gracilior* inferior however to the water-melons of Persia and neighbouring countries. Again *Cucurbita Pepo* has a special Anatolian sub-species, *turcica*, representing the greatest variety of forms known in Europe, in addition to which there occur peculiar bushy forms. It is significant that the Anatolian forms of all the various cucurbit species, including the cucumbers, are characterized by the same group of botanical features, *viz.*, that associated with the type *gracilior*. New botanical groups are established within the respective species, each consisting of a number of botanical varieties, all of which are endemic and are described; each has a clear regional distribution, which is indicated. It is clear that Anatolia is the primary centre of origin of the melons and an important secondary centre for the pumpkins and watermelons. Even the cucumbers which penetrated from India, have to a certain extent evolved and produced a characteristic local type.

As regards the other vegetables great variability was found among the beets, not only in morphological but in biological and ecological characters; a whole group of orange-coloured beets is endemic in central and eastern Anatolia, a group having exceptional interest as breeding material. Again in the case of carrots, all the characters, and all the forms known to exist in the cultivated carrot are concentrated in Anatolia, both those associated with the Asiatic and with the European groups, both of which are represented; moreover, a much greater range of variation has been disclosed by this material than was known to exist in the carrot and new possibilities in breeding have been opened up. In addition to typical European and typical Asiatic types, all possible intermediates are found not in respect of one character alone but in respect of all distinguishing characters. Investigation has shewn that the Asiatic carrots are lacking in plastids, the pigment being in the cell sap, whereas the European group owes its colour entirely to the plastids; hybrids between them contain pigment of both kinds and so also do the intermediate forms occurring in Anatolia. In addition there is an

endemic group distinguished by the very large dimensions of the whole plant and more nearly allied to the wild sub-species *Daucus Carota* subspecies *Maximus*. This new group, named *Adanensis*, also contains purple and yellow forms corresponding to those occurring in the cultivated group. The concentration forms in Anatolia is evidently the result of the overlapping in this area of the two wild sub-species *D. Carota* subspecies *Carota* and *D. Carota* sub-species *Maximus* from whose hybridization the European group of cultivated carrots has evidently arisen. Anatolia is thus regarded as the primary centre of origin of the European group as well as a secondary centre of originl of the cultivated carrot as a whole. Although nearly the whole range of variation of carrots found in Anatolia, with the exception of the endemic Anatolian forms, occurs also in the various countries of the Soviet Union, the Anatolian populations are more regular in form and size of root and are therefore useful for breeding. Some of them contain a valuable essential oil, lemonene, and most have the advantage of being drought-resistant and, finally, their richness in characters makes these carrots an invaluable starting point for genetic studies.

The Anatolian cabbages are characterized by pronounced response to the influence of length of day, to which the European cabbages are more or less neutral. The cabbages of Anatolia are more drought-resistant than any yet known ; they are very susceptible however to *Peronospora brassicae*. In addition to cauliflowers there are specimens of the headless cabbage (var. *acephala*) and hybrids between them showing vegetative segregation. Hybrid forms between *acephala* and the headed cabbage were also found Many of the forms of the cabbage group are of a semi-cultivated type and some are endemic ; the variety of forms of the oriental group is greater than that in neighbouring countries to the west and there are indications that the Anatolian cabbages form an ancient group in the origin of which the Syrian group has participated. They contain many forms and characters of value to Soviet breeders and will be used both in analytical and synthetic breeding.

Quantities of wild olives are found growing even in most unfavourable situations and poor mountain soils. There is also an abundance and variety of figs, of wild species and forms of pears, plums, cherries, almonds, hazels, walnuts, chestnuts, pistachio, the kernel pine, *Rubus*, *Ribes* and *Vaccinium*; and there is an endemic wild species of vine, *Vitis orientalis*. The Smyrna fig, the North Syrian pistachio and certain groups of the cherries, pears, plums and almonds are endemic. All these wild fruits and nuts are used by the inhabitants and often the inferior ones are removed to leave room for the best specimens ; in this way they have come gradually into cultivation, and various degrees of the process are to be seen at the present time.

The peculiar characteristics of the flora of Anatolia are explained when it is realized that this is the only country where the continents of Asia, Africa and Europe meet. It is the meeting place of different types of allied plants, the oriental anthocyanin carrot and the wild carrot, the cultivated and wild vine, the large and small-seeded forms of many leguminous plants, hence intermediate forms or often new types altogether have

resulted. The wide range of ecological conditions in the country and the low degree of purity of the crops have favoured the preservation of the greatest possible number of these new forms. Yet most field and vegetable crops are remarkably uniform as regards the character and quality of the seeds, evidence of a deliberate selection on the part of the agriculturists of the past. All these reasons have contributed to make Anatolia the source of many of the most valued varieties cultivated in Europe.

The volume under review terminates with a bibliography of 27 pages, an index of Turkish, Latin, Russian and geographical names and an extensive French summary It is provided with an excellent map of the country and is unusually well printed and illustrated.

NOTE

SOIL DEFICIENCIES AND PLANT DISEASES : IMPERIAL BUREAU OF SOIL SCIENCE TECHNICAL COMMUNICATION No. 31

Technical Communication No. 31 of the Imperial Bureau of Soil Science, Rothamsted, "Soil Deficiencies and Plant Diseases" deals with the border line of plant physiology and soil chemistry. Dr. Jacks and Miss Scherbatoff have collected and summarised the relevant facts from some hundreds of papers and provided a bibliography of 367. This bulletin does not deal with the phenomena associated with nitrogen, potash or phosphatic starvation, but with the pathological conditions which have been ascribed to deficiencies of the so-called minor elements, notably manganese, iron, magnesium, boron, sulphur, copper and zinc.

A handy table of deficient elements, main crops affected and numerical references to the main bibliography considerably enhance the utility of the bulletin (Ed).

GIPD—M647 IC of AR—10-6-35—800.

ORIGINAL ARTICLES

ORGANISMS ASSOCIATED WITH SUGARCANE MOSAIC AND THEIR RELATION TO THE MOSAIC VIRUS

BY

S. V. DESAI, B. Sc., PH.D., D.I.C.,

Sugarcane Mycologist, Pusa

(Received for publication on the 11th February 1935)

I. INTRODUCTION

Various types of organisms have been reported to be the causative agents of plant virus diseases. Nelson [1923] attributed the virus diseases of bean mosaic to a protozoan, and Eckerson [1926] described minute flagellate organisms appearing after 24 hours in mesophyll cells of leaflets opposite to those inoculated with filtered tomato mosaic virus. Several workers have associated bacteria with virus diseases. Nelson [1932] described small cocci in mosaic-infected beans. He considered from the results of his bacteriological studies together with the result of seed transmission tests that there was a close association of the coccus with the virus whatever the latter may be. However, inoculation experiments with these organisms were unsuccessful in reproducing the disease.

Bewley [1931] advanced a suggestion from his observation of constant association of pleomorphic bacteria with tomato mosaic that the virus was a kind of bacteriophage living on the bacteria and parasitizing the plant under suitable conditions. Swezy and Severin [1930] reported the association of rickettsia-like micro-organisms with curly top of sugar beet. They were unable to differentiate on morphological data between the two types of organisms which they found—but one type was found to pass the Berk feld filter while the other did not. Filterable ones were found only in the infective insect vectors, the others occurring both in healthy and infective insects.

Takahashi and Rawlins [1932] have put forward evidence of the existence of minute rod-shaped bodies in sap containing virus by examining the clarified juice of tobacco mosaic by polarised light. Melhus [1922], Bouquet [1916] and Smith and Bouquet [1915] have associated bacteria with the causative agents of

plant virus diseases. Brehmer and Barner [1930] and Brehmer [1931] described yellowish green, oval, amorphous bodies which they consider are organisms belonging to a hitherto unknown systematic group of the Archimycetes allied to Plasmodiophoracæ.

Desai [1933] evolved a method of cultivation of the virus principle *in vitro* and gave evidence of virus principle being related to forms of bacteria usually found associated with tomato mosaic and photographed the minute flagellate granules after mordanting them and staining with flagella stains.

The present work was undertaken to find out whether similar organisms were associated with sugarcane mosaic, and if so to find out their relationship to the mosaic virus.

II. Isolation

A. By tissue implantations

The first method tried to obtain cultures of bacteria associated with sugarcane mosaic was by tissue implantations. For this purpose the stem of sugarcane having mosaic disease was thoroughly sterilized by repeated washing with mercuric chloride and alcohol. A sterile cork borer was then inserted in the internodes near the growing point and an inch of the plug was quickly transferred to an agar slant directly from the cork-borer. The cork-borer was plugged with cotton at the top end to prevent infection from outside.

The media generally used were sugarcane extract broth and agar of the following composition :—

Broth	Agar
0·5 grm. dipotassium phosphate	
0·2 grm. magnesium sulphate	
3·0 grms. Marmite	1.5 per cent agar was added for agar medium.
1·0 grm. Mannite or cane sugar	
1000 c.c. of sugarcane leaf extract	
The extract was prepared by steaming 200 grms. of sugarcane leaves in 1000 c.c. of water for 30 minutes and filtering.	

After adjusting the pH to 7.8 the media were sterilized at 120°C. for 30 minutes.

The culture tubes were incubated at 30°C. for long periods after sealing them with paraffin wax. In some cases fungi and bacteria were found growing abundantly in a couple of days time. Some tubes remained sterile for more than six months by which time the agar had usually dried up, and in some tubes thin growth of bacteria developed after a month or so. These tubes were still moist with water of condensation while the tubes which ultimately remained sterile for six months were dry and without any water of condensation at this stage though sealed up with paraffin wax.

Moisture appeared to influence the growth. Sub-cultures from these bacterial growths were difficult to cultivate on agar and generally no growth was visible in the transferred slants. Plates poured were also devoid of visible colonies but occasionally very minute colonies were noticed. The colonies were imperceptible with the naked eye but could be viewed under a low magnification.

Visible growth was obtained only after sub-culturing such isolated colonies six to seven times. Usually after 10 transfers typical, easily cultivable bacterial growth was established. The cultures by this method were obtainable in 60 per cent of cases tried, in 30 per cent of cases contaminations occurred, while in 10 per cent of the cases the tubes remained sterile till they dried up.

B. By broth transfers

Methods of serial broth transfers have been recommended by Hadley [1931] to demonstrate the presence of the filterable forms of bacteria. Sugarcane extract broth as previously described was ordinarily used.

The juice of mosaic leaves 0.2 to 0.5 c. c., filtered through L_3* filter candle, was introduced in broth tubes and incubated for 5 days. No visible growth of any organism was found in the tubes. These tubes were filtered through a sterile L_3 filter candle and 0.2 to 0.5 c.c. of the filtrate put in fresh series of broth tubes. The process of filtration and transfer to fresh series of broth tubes was carried on until some visible sign of growth was observed in the culture tubes. This usually occurred after about 7 to 8 passages when a slight opalescence developed. The juice of the healthy sugarcane leaves under the same treatment failed to show similar phenomenon even after 20 such passages. The growth in broth at this stage if spread on agar failed to show visible colonies. The growth in serial passages after opalescence became progressively visible and after a passage or two became cultivable on the sugarcane extract agar. The growth on agar consisted of minute pin point colonies barely visible to the unaided eye. Usually after ten to eleven passages the growth became quite perceptible, with flakes ; and grew on agar in a uniform layer dotted with plaques, like a bacterial culture associated with its bacteriophage.

* Finer grades of filter candles L_3 to L_{13} were used and they too gave similar results.

The same type of growth was observed in 90 per cent of the serial passages carried out while in four per cent the culture tubes got contaminated and in six per cent of the cases the culture was not obtainable even after 20 serial passages. Simultaneously with each of the series with mosaic juice a similar series with juice of healthy leaves was carried out and in 94 per cent of the cases no growth was obtained, in two per cent of the cases contamination occurred and in four per cent of the cases the typical bacterial culture was obtained. It may be that the organisms were present in the plants which were considered healthy, the disease being present with masked symptoms, or in view of the constant presence in the laboratory of these organisms these four per cent of the cases may be better attributed to contamination with the organisms.

C. By ageing the leaf juice

The juice of the mosaic leaves was filtered through L_3 filter candle and incubated under sterile conditions after sealing in ampoules. The juice ordinarily remained clear for more than a year but some times after a couple of months slight opalescence developed which continued increasing till definite growth was visible. The sealed tubes were then broken and the juice implanted on agar surface and in broth. The agar showed some growth if the juice was cloudy, otherwise with slightly opalescent cultures no visible growth was observed on agar. In broth the growth was always obtained and appeared to be of the same type as obtained by successive passages previously described.

In twenty per cent of the sealed ampoules the growth described above developed and 80 per cent of the ampoules remained unchanged. But if serial transfers were made from these tubes in broth, very frequently similar growth was observed after three serial transfers.

III. Cultural behaviour

Cultivation of the organisms from the mosaic tissues was found to be difficult. Cultures were unaccountably lost and failed to show visible growth. Transfers of such unviable culture in broth often brought about slight opalescence but transfers from these showed no growth on various agar media. Gelatin media also proved equally unsuitable. Broth cultures, and their transfers also, often remained quite limpid with no visible growth. Various broth media were tried to fix upon the most suitable one. But there was little to choose as almost all media behaved similarly. Sugarcane extract broth with 0·2 per cent lithium chloride was found to be the best. The morphological forms in broth were mixed and variable. From very minute cocci to well sized rods were common when visible growth occurred. If only opalescence was present, no rods or cocci were

found but minute granule-like forms which could not be differentiated from stain particles or protein droplets of the media were prominent. Flagella staining at this stage did not differentiate these granules from usual media debris.

Attempts were made to study the cyclostages of the bacteria by repeated serial passages but no regular behaviour was observed as the visibly cultivable stage was found to be very short if transfers were continued in broth. Acidic and neutral culture media did not improve matters.

The relative lengths of various cyclostages of these organisms were compared with those of Shiga Bacillus (in which filterable forms and other cyclostages are well studied). This brought out the fact that these organisms were most stable in the virus cyclostage :—

TABLE I

	Bacterial cyclostage "R. & S." stage	Filterable cyclostage "G" forms	Virus cyclostage invisible growth stage
Organisms associated with mosaic virus .	4	8	88
Shiga Bacillus	78	20	2

Thus during the life cycle of 100 days these organisms had the bacterial cyclostage for only four days as against similar cyclostage of 78 days in Shiga cultures, filterable or "G" form cyclostage for eight days as against similar cyclostage of twenty days in Shiga cultures and invisible virus cyclostage of 88 days as against similar cyclostage of only two days in Shiga cultures. This accounted for the rapid transformation and unstable bacterial cyclostage of the virus organisms and their peculiar pleomorphic growth. It also explained why it was difficult to study the behaviour of the organisms by ordinary bacteriological technique and biochemical reactions.

Cultivation of the organisms on agar and other solid surface offered still greater difficulty than cultivation in broth. Virus or invisible cyclostage was not cultivable on agar and if continuous transfers were made by transferring water of condensation, though no growth was visible no growth developed and transfers to a series of broth tubes ultimately failed to reveal the presence of the

organisms. It was therefore concluded that cyclostage was unable to multiply on solid media, but with the change from " virus " cyclostage to opalescent forms and to " bacterial " stage the cultivation on agar was progressively successful. The cyclostage which gave opalescence in the broth media usually gave a growth of minute colonies invisible to the unaided eye but distinguishable under low power objective. The broth cultures which were distinctly cloudy gave uniform bacterial growth studded with minute round clear areas similar to bacteriophage plaque or minute colonies of different type than the ordinary smooth growth. These areas appeared to be devoid of any growth in the beginning but on ageing fine annular growth began to be visible. The growth was of different type to the surrounding one. Transfers from such plaques usually failed to grow on agar or any other solid media, in broth they sometimes produced opalescence, but mostly no change and rarely visible clouding typical of the organisms. The transfers from bacterial growth were easily cultured on solid as well as on broth media but sometimes the transfers appeared sterile without any clouding or colony formation, and in such cases it was extremely difficult to recover the culture from such culture tubes. If 24-hourly transfers were carried out the cultures soon got lost but with fortnightly or monthly transfers the culture could be maintained with slight difficulty. The cultures of minute colonies were not stable in that phase and more often failed to give growth on transfers in solid or broth media. Sometimes this phase or cyclostage was found to give the same type of growth on solid agar for some time and vanishing completely afterwards on further transfers. This cyclostage developed if Chamberland candle filtrates from the suspension of the old culture of the organisms were spread on agar surface and that was the only reliable source of this filter-passing visible cyclostage giving minute colonies. The culture of these minute colonies could be maintained as such for a few transfers after which the culture either failed to develop visibly or gave uniform growth composed of large colonies studded with minute plaques.

IV. Morphology and biochemical reactions

From the foregoing description it would be evident that the culture of these organisms was usually a mixture of different cyclostages but at the same time each cyclostage could be obtained in more or less pure form by suitable technique.

The life-history of the organisms may be subdivided into following well defined cyclostages:

1st: Filterable and invisible (virus), 2nd: Filterable and visible (" G " type culture of Hadley), 3rd: Unfilterable and visible (Bacterial), 4th: Spore form.

The morphological and biochemical behaviours of each cyclostage were studied as far as possible separately and are given below :—

1st : Filterable and invisible cyclostage (virus). This cyclostage gave no visible growth in agar or broth media, but on repeated transfers slowly passed into the second cyclostage of filterable but visible type of cultures similar to those described by Hadley.

2nd : Filterable and visible (" G " type culture of Hadley). These forms formed minute colonie on solid media and gave opalescent and hazy growth in broth media. This stage very often reverted to virus stage. The individual colonies almost invariably reverted but mass cultures steadily passed on to the next cyclostage of unfilterable forms. The organisms in this cyclostage were very minute and just resolvable with the highest power. Very fine granules were visible on staining with usual aniline dyes. In the impression films, obtained by touching the cover glass on to the colonies of the organisms, minute coccus forms predominated and very occasionally small rod-like forms could be differentiated. On staining such films with Zetnow's flagella stain a few minute rods with one long flagellum could be observed ; the number of such organisms, however, was not large. Fine granules mostly in clusters were preponderant. Most of the forms of this cyclostage were Gram negative.

The biochemical reactions were negative in almost all biochemical media, sugars were not fermented, nitrates were not acted upon, hydrogen sulphide, indol, and ammonia were not formed. Litmus milk turned slightly acidic without peptonization or coagulation. Gelatin was not liquefied, growth on potato was not visible and no diastatic action could be demonstrated. Pin-point colonies often developed after prolonged incubation but were too small to give definite information as regards colour and type of growth.

Spore formation was conspicuously absent, and aged cultures though viable failed to retain acid fast stain. Generally such cultures reverted to virus cyclostage and rarely into unfilterable cyclostage. Cultures could not be maintained in this stage for long and could only be re-isolated from the Chamberland candle filtrates of ordinary bacterial cyclostage.

3rd : Unfilterable and visible (Bacterial). Biochemical reactions of the bacteria which ultimately developed from the filterable stage gave negative reaction with almost all biochemical media. No acid or gas was formed in sugar media, nitrate was neither reduced nor formed. No ammonia developed in peptone. Indol and hydrogen sulphide were not formed. Litmus milk turned slightly acidic and peptonization slowly took place with clear yellowish upper layer, slightly reddish middle layer and bluish bottom layer. Gelatin was very slowly liquefied. The liquefaction was crateriform. The organisms were strictly ærobes, and gave a mealy growth with a dirty colour on potatoes. Diastatic action was absent.

The organisms were generally Gram negative but some times retained the Gram stain.

The size of the organism varied from 0·1 μ to 0·7 μ in thickness and 0·3 μ to 3·5 μ in length.

Flagella. The organisms were usually found to have a single polar flagellum but sometimes 3 to 5 flagella were observed occurring at both the polar ends. Peritrichous flagella were also noticed to occur and aged stock cultures occasionally developed this form. In some cultures non-motile forms without any flagella were obtained. This condition was usually rare and preceded the spore formation.

The organisms took acid and basic stains readily. The slime and capsules were absent.

4th : Spore stage. Very rarely spores were observed to occur and then the culture usually assumed a wrinkled appearance on the slant. The spores were oval and measured 0·3 to 0·5 μ in breadth and 0·8 to 1·2 μ in length. Experiments to find the conditions governing the spore formation did not give any results as all methods tried failed to stimulate spore formation. It appeared that under adverse circumstances the culture readily reverted to filterable and virus cyclostages.

Thus the organisms isolated differed in cultural and biochemical reactions from all known bacteria. Some resemblance to organisms isolated from the diseased tissues of tomato mosaic by Desai [1933] was observed but points of difference were distinct and many.

V. Serological reactions

(*a*) *Why carried out*

The repeated failure to induce the disease by the isolated organisms, their filterable and virus forms, suggested the investigation of the relationship of these to mosaic virus by serological tests.

These tests are all based on the principle that a serum containing antibodies produced against some organisms previously injected into the animal from which the serum is obtained, will give a positive reaction only with the organisms used in the injection or with some closely related type. Through the relative specificity of these reactions it is possible to study the relationships between different organisms and strains, and this method was utilized to study the inter-relationship between the bacteria, its filterable forms, and the mosaic virus. The tests were carried out by the method recommended by the Sub-Committee on serological methods of the Society of American Bacteriologists [1928].

The inoculations were made with cultures of bacteria, their filtrates, the virus (juice of mosaic leaves filtered through candle) and the juice of healthy leaves similarly treated.

(b) *Purity of antigens*

To obtain reproducible results it is highly essential that the material (the antigen) for injection into rabbits should be carefully purified and standardized. Each antigen was scrupulously tested for purity and guarded against chances of contamination. The method for obtaining each is given below :—

1. The virus antigen.—The young sugarcane leaves of Co. 213 showing heavy mottling were used throughout the experiment. The standard virus juice was prepared by crushing 50 grms. of leaves in 50 grms. of water and extracting 45 grams of the juice from the mixture through a cheese cloth. This juice was repeatedly filtered through the same filter paper till it gave clear brownish filtrate. The filtrate was then filtered through L_3 filter candle under 5 lbs. pressure into sterile tube. The glazed portion of the filter candle was wrapped in non-absorbent cotton wool, inserted into wide test tubes and the assemblage was sterilized by dry heat at 160°C. for 2 hours. The filtration was carried out according to the method of Martin. The candle was removed and the tube plugged with sterile cotton. The antigen was prepared fresh for each inoculation. The filtered juice remained clear and sterile.

2. Healthy juice antigen.—The material was prepared by the procedure employed for virus antigen from healthy sugarcane leaves of the same age as the mosaic ones.

3. Bacterial antigen.—The bacterial antigen was prepared by suspending the 48-hour growth on nutrient agar in sterile physiological saline. The growth was removed by platinum loops from the surface without disturbing the agar. The suspension was filtered through a sterile filter paper, standardized by opacity measurements and used immediately.

4. Filtrate antigen (for filterable forms).—The suspension of the bacteria prepared as mentioned above was filtered through sterile L_3 filter candle. The filter candle was assembled as previously described so that every possibility of chance contamination from air was avoided. The filtrate was prepared fresh each time and used immediately.

Scrupulous care was taken in handling various material used for the antigenic purpose and from beginning till the end kept under sterile condition.

(c) *Method of inoculation*

The antigens were injected into the marginal ear vein of the young rabbits by taking up the antigens in sterile Record syringes. A separate syringe was used for each antigen, and hands and everything used for inoculation were scrupulously sterilized before each inoculation. The amount of the dose was increased slowly

till the maximum was reached after five to eight inoculations. The experiments were repeated four times with different batches of rabbits at different times and the procedure employed remained the same throughout. The results obtained were similar. The detailed history of the immunization of one batch is given here for information, the others being omitted to save space :—

TABLE II

Date (1934)	I Mosaic leaf juice		II Bact. sus.		III Filtered Bact. sus.		IV Healthy leaf juice		V Bact. sus.		Remarks.
	Wt. lb. oz.	Dose c.c.	Wt. lb. oz.	Dose c.c.	Wt. lb. oz.	Dose c.c.	Wt. lb. oz.	Dose c.c.	Wt. lb. oz.	Dose c.c.	
19th Feb.	4-7		3-2		4-12		3-0		3-10		
20th ,,		2.0		0.2		2.0		2.0		0.4	
26th ,,	4-11		3-10		4-14		3-7		3-12		
27th ,,		2.6		0.4		2.6		2.6		0.6	
5th March	4-12		4-0		5-0		3-12		Died * 28th Feb. 1934.		
6th ,,		3.0		0.6		3.0		3.0			
12th ,,	4-11		4-2		5-2		3-14				
13th ,,		3.6		0.8		3.6		3.6			
19th ,,	4-12		4-3		4-12		4-2				
20th ,		4.0		0.9		†		4.0			
26th ,,	4-8		4-1		4-8		4-2				
27th ,		4.0		1.0		3.8		4.5			
3rd April	4-10		4-3		4-3		4-8				
3rd ,,		4.5		1.2		4.0		4.5			
9th ,,	4-13		4-1		4-12		4-8				
10th ,,		4.5		1.5		4.5		4.5			
17th ,,	5-0		4-1		5-0		4-6				
20th ,,	Bled		Bled		Bled		Bled				

* Broncho-pneumonia and peritonitis—left lung badly affected, right lung partly. Serosanguineous fluid peritoneal and pleural cavities.

Swelling in ear : production of fibres in the vein due to irritation, consequent blocking of vein, evident only in the vein which received the inoculation.

† Drooping and bent towards the right side as if it were paralysed. Inoculation suspended.

(*d*) *Tests and their reactions*

Ten days after the last inoculation, the rabbits were bled from ear vein or by the heart puncture if more blood was required. The serum of each rabbit was

prepared separately and stored in an ice chest. The following reactions of the sera were studied :—

1. Agglutination of the organisms of sugarcane mosaic.
2. Precipitation of mosaic juice, filtrate of the organisms and healthy juice.
3. Hemolytic properties.
4. Complement fixation.

1. Agglutination.—The clumping or agglutination of bacteria under the influence of an immune serum is an important and well-known method for the differentiation of bacteria. The test is based upon the observation that the injection of bacteria into an animal causes the production of antibodies called agglutinins, which have the power of clumping (agglutinating) suspension of the same or closely related organisms when brought in contact with them under the proper conditions. Conversely, if one organism is agglutinated by different sera then the different organisms used for immunizing the rabbits have a very close relationship with the organism in question.

This test has been carried out very often and has always given similar results. The 48-hour old cultures were used for the test and these were suspended in physiological saline and filtered through filter paper. Light suspensions gave distinct and well defined end points. The sera were diluted in progression and mixed with equal quantity of the suspension in agglutination tubes. The tubes were kept at 56°C. for 30 minutes and in the ice chest overnight. The readings were taken next morning. Keeping longer at the high temperatures was not found to be useful.

Results of two typical experiments are given below :—

TABLE III

Agglutination reaction

Rabbits batch No. 2, bled on 19th December 1933 immunized by eight inoculations

Sera dilutions	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
Serum I virus .	++++	++++	++++	+++	++	+	±	—
Serum II filtrate	++++	++++	++++	++++	++++	+++	+++	++
Serum III organism	++++	++++	++++	++++	++++	+++	+++	++
Serum IV healthy juice	—	—	—	—	—	—	—	—

TABLE IV

Agglutination reaction

Rabbits batch No. 3, bled on 20th April 1934, immunized by eight inoculations as given in Table II

Sera dilutions	1:25	1:50	1:100	1:200	1:400	1:800	1:1600	1:2400	1:3200	1:6400
Serum I virus . .	++++	++++	++++	++++	++++	+++	++	+	—	—
Serum II filtrate . .	++++	++++	++++	++++	++++	++++	+++	++	—	—
Serum III bacterial .	++++	++++	++++	++++	++++	++++	+++	+	—	—
Serum IV healthy juice .	+++	++	++	++	—	—	—	—	—	—

The signs employed to record the results have usual serological significance as follows :—

++++ Complete agglutination *i.e.* sediment at the bottom of the tube with completely clear supernatant fluid.

+++ Complete agglutination. Sediment at the bottom and sides. Supernatant fluid quite clear.

++ Partial agglutination. Appreciable sedimentation at the bottom and sides. Supernatant fluid slightly turbid.

\+ Positive agglutination. Slight sediment at the bottom and sides. Supernatant fluid slightly clearer than the controls.

± Doubtful agglutination. Slight sediment on sides only and visible with lense.

— No agglutination. No sediment in the suspension. The suspension as turbid as the controls

2. Precipitation test.—This test was carried out to see if the virus and other antigens were precipitated by their homologous and heterologous antisera. For this purpose increasing dilutions of the sera were brought in contact with the antigens and the formation of visible precipitation noted. Each serum was put up against virus (mosaic leaf juice) filterable forms (filtrate of organisms) and healthy leaf juice and incubated in a water bath at 56°C. for 4 hours. The tubes were then transferred to an ice chest and kept overnight. Results were read following morning.

The result of a typical test is given below :—

TABLE V

Precipitation test, dated 7th July 1934

Sera dilutions		1:20	1:50	1:100	1:500	1:1000
Serum I virus	V.	+	±	—	—	—
	F.	±	±	—	—	—
	HJ.	±	±	—	—	—
Serum II *filtrate*	V.	+	±	—	—	—
	F.	+	±	—	—	—
	HJ.	—	—	—	—	—
Serum III healthy juice	V.	±	±	—	—	—
	F.	—	—	—	—	—
	HJ.	+	±	—	—	—
Controls . .	V.	—				
	F.	—				
	HJ.	—				

It would be seen from these results that the reaction was not very satisfactory. Modification in procedure did not help in making the doubtful precipitation definite.

Taking only definite precipitation as indicative of the affinity, the relationship between virus and filterable form was brought out. Virus serum reacted only with its own antigen and doubtfully with filtrate and healthy juice. Filtrate serum reacted definitely positive with virus and filtrate antigens and definitely negative with healthy juice while healthy juice serum reacted with its own antigen and doubtfully with virus antigen. In the more recent work of this nature on tobacco mosaic virus by Birkland [1934] it has been found that plant protein in the juice used for immunization interferes in the clarity of the reaction.

The plant proteins present in the juices used for immunization were estimated by finding total nitrogen and arriving at the amount of proteins present in the juices approximately :—

	Mosaic	Healthy
	Per cent	Per cent
Amount of nitrogen	0·01	0·01
Amount of protein calculated	0·06	0·06

It would appear from these that the amount of plant proteins in this case was too small to interfere in the reaction ; again qualitative tests of protein (namely biuret, xanthoproteic, Adamkeivicz, and Millon) were very faint in the juices employed for immunization.

3. Hemolytic properties.—The hemolytic action of the various antigens—namely the virus, filterable forms, bacteria and healthy juice—was investigated. For this purpose 0·25 c.c. of each of the antigens was mixed with 0·25 c.c. of unsensitized sheep's red blood corpuscles and 0·5 c.c. of saline in quadruplicate tubes and kept at room temperature for 30 minutes and at temperature of 56°C. for another 30 minutes. The tubes were then removed and stored in ice box overnight, the reaction being observed the following morning. The results were negative, that is the R. B. Cs. were not hemolysed.

4. Complement fixation.—Complement fixation test is based upon the observation that the combination formed between an antigen and its specific antibody has the property of fixing complement during the reaction. On the basis of this general law complement fixation can be used to detect the union of an antigen with its homologous or specific antibody. The test for such fixation is performed by placing together antigen, antibody and complement in suitable proporation as determined by previous titration and subsequently testing for the presence of free complement. If complement is not fixed, it indicates that the antigen and the antibody do not have power to unite or in other words, the antigen and the antibody are not specifically related. On the other hand the fixation of the complement in the mixture indicates that the antigen and antibody have combined owing to their specific affinities.

The test is very delicate and specific but there are cases in which, though the union of the antibodies with the antigen does take place, the complement is not fixed in the reaction. Thus whereas the positive fixation is a very strong proof of the union of antibody with the antigen the non-fixation of complement does not as a rule preclude the possibility of the union between antibody and the antigen in pure culture studies.

The test is much more specific than the agglutination test, requires careful attention to details and the preparation of number of accurately standardized serological reagents.

The complement fixation test was carried out with both heat stable and heat labile antibodies and the results of these tests are given below :—

The tests were carried out by mixing :

·25 c.c. of diluted sera (1 : 10)

·25 c.c. of the antigen

·25 c.c. of the complement of appropriate strength in minimum hemolytic doses (M.H.D.).

The mixture was kept at room temperature for 30 minutes followed by a temperature of 56°C. for 30 minutes. At the end of this period 0·25 c.c. of standard sensitized red blood corpuscles (R. B. Cs.)—were added and the trays containing the tubes kept at 56°C. for 30 minutes. The trays were then withdrawn from the water bath and kept overnight in the ice chest. The readings were taken next day.

The symbols and abbreviations used have their usual serological significance.

TABLE VI (*a*)

Complement fixation test with the unheated sera

Strength of the complements	Virus serum I against				Filterable form serum II against				Bacterial serum III against				Healthy juice serum IV against			
	V.	F.	O.	HJ.	V.	F.	O.	HJ.	V.	F.	O.	HJ.	V.	F.	O.	HJ.
8 M. H. D.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6 M. H. D.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5 M. H. D.	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4 M. H. D.	+±	—	—	—	±	—	±	—	—	—	—	—	—	—	—	—
3 M. H. D.	+±	±	±	—	+	±	+	—	—	—	—	—	±	—	—	±
2 M. H. D.	++	±	±	±	+±	±	+±	—	++	—	+	—	±	—	±	±

TABLE VI (*b*)

Controls

	Serum controls				Antigen controls			
	I	II	III	IV	V.	F.	O.	HJ.
5 M. H. D.	—	—	—	—	—	—	—	—
4 M. H. D.	—	—	—	—	—	—	—	—
3 M. H. D.	—	—	—	—	—	—	—	—
2 M. H. D.	—	—	—	—	+±	—	—	±

TABLE VII (*a*)

Test carried out with the heated sera

Strength of the complement	Virus serum I against				Filterable form serum II against				Bacterial serum III against				Healthy juice serum IV against			
	V.	F.	O.	HJ.	V.	F.	O.	HJ.	V.	F.	O.	HJ.	V.	F.	O.	HJ.
10 M. H. D. .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8 M. H. D. .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6 M. H. D. .	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5 M. H. D. .	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4 M. H. D. .	±	—	—	—	±	—	+	—	—	—	—	—	—	—	—	—
3 M. H. D. .	+±	—	±	—	+	—	+	—	±	—	±	—	—	—	—	±
2 M. H. D. .	+±	±	+	±	+±	±	++	—	+±	+	+±	—	±	—	—	±

TABLE VII (*b*)

Controls

	Serum controls				Antigen controls			
	I	II	III	IV	V.	F.	O.	HJ.
5 M. H. D.	—	—	—	—	—	—	—	—
4 M. H. D.	—	—	—	—	—	—	—	—
3 M. H. D.	—	—	—	—	—	—	—	—
2 M. H. D.	—	—	—	—	+±	—	—	±

Interpretation of the results of the complement fixation test indicated that the mosaic leaf juice (that is virus) has a distinct antigenic property as compared to healthy leaf juice. Mosaic juice was partly anticomplementary while healthy leaf juice was very slightly anticomplementary. Complement fixation with virus antigen was greatest when it was put up against its own serum ; less with filterable form serum, and organisms serum ; and least (even less than control) with healthy juice serum.

Filterable forms antigen when put up with different sera failed to fix any complement.

Organisms antigen fixed complement slightly when put up with its own serum and that of filterable form, otherwise the reactions were negative.

Healthy juice did not fix any complement even when put up with its own serum, thus showing a lack of antigenic substance in the leaf juice. This indicated that the antigenic substance in the mosaic leaf juice was the virus.

Antibodies were heat-stable, that is they could be heated without inactivation upto the temperature of 56°C. for half an hour.

The complement fixation was not much but it was sufficient to show the relationship between the virus, filterable forms and the organisms and supported the agglutination tests which were more definite. The difference from the reactions of the healthy juice serum was very marked.

The tests were carried out at Haffkine Institute, Bombay, by kind permission of Col. S. S. Sokhey, Director, Haffkine Institute. Dr. Wagle, Assistant Director, Haffkine Institute, greatly helped me in carrying out the tests proper for which I am much indebted to him. The results of other similar tests are omitted to save space.

(e) *Effect of sera on infectivity of the mosaic leaf juice*

These experiments were carried out to see what action was induced by the various sera on the infectivity of the mosaic leaf juice. The leaf juice filtered through a cheese cloth was used for these experiments.

The standard mosaic leaf juice was mixed with one tenth of its volume of appropriate serum and the mixture well shaken and inoculated into test plants within five minutes. Controls were also inoculated with mosaic leaf juice similarly diluted with water.

Scrupulous care was taken in inoculating the plants. Bundles of entomological needles mounted on corks were carefully sterilized and all other articles used were also sterilized. The hands were washed with an antiseptic in between the inoculations. The mixtures were slightly pricked with the needles in base of the leaves.

The appearance of mosaic in the plants was noted. The results of one of these experiments are given below :—

TABLE VIII

Treatments	Pots	No. of inoculated plants	Date of inoculation	No. of plants showing mosaic			
				30th Sept. 1933	2nd May 1934	10th May 1934	21st May 1934
Control (mosaic leaf juice).	9	20	23rd April 1934	0	4	11	18
Mosaic leaf juice+ healthy juice serum.	10	16	,,	0	3	7	11
Mosaic leaf juice+ mosaic serum.	10	20	,,	0	0	0	0
Mosaic leaf juice+ filtrate serum	10	20	,,	0	0	0	0
Mosaic leaf juice+ bacterial serum.	10	18	,,	0	0	6	12

These experiments definitely showed that the mosaic juice was inactivated or neutralized by its own serum and further that such inactivation was also caused by filterable form serum. The healthy juice serum as well as bacterial serum had no action on the virus. Thus a definite proof of the similarity between the filterable forms of the bacteria and the virus was obtained, as the two above-mentioned antigens produced such antibodies that they united and neutralized the virus. That animal serum as such has no such effect was clearly shown by other sera being unable to neutralize the virus.

Further experiments were conducted with the experimental plants of this series.

1. Some of the non-mosaic plants from each of the series were ground up and used for inoculating a further batch of plants to find out whether the symptoms of the mosaic disease were masked in these plants and the plants had become carriers of mosaic with mosaic virus multiplying in them. It was found that the non-mosaic plants of all the series failed to infect the plants inoculated with their juices showing thereby that the masking of symptoms did not take place and that the plants were really mosaic free.

2. Two of the plants showing no mosaic were reinoculated after 45 days with the mosaic leaf juice to find out whether the plants were susceptible, or an immunity inherited or artificial had developed in the plants.

When the plants were finally examined it was found that the non-mosaic plants from the control series did not take infection even on reinoculation, showing a kind of inherent resistance, while the non-mosaic plants of the virus-serum mixture series took infection on reinoculation very readily, showing that though the plants were susceptible the virus was neutralized *in vitro* in mixtures used for first inoculations. Non-mosaic plants of the filterable form serum mixture series when reinoculated with the mosaic leaf juice failed to take the infection and it might be due to an acquired resistance. Further experiments are necessary to come to any definite conclusions in this respect as the plants tried were only two.

Further experiments were conducted to see if the sera conferred immunity to the plants if they were inoculated into them for this purpose. The sera diluted ten times with sterile saline solution were inoculated into a batch of plants and the

standard mosaic leaf juice was pricked into them after 10 days. The results of this experiment are given below :—

TABLE IX

Treatments	No. of pots	Date of inoculation	Date of M. L. J. inoculation	Mosaic appearance 27th May 1934
Virus serum	2	23rd April 1934		None
Virus serum followed by mosaic leaf juice after 10 days	8	,,	3rd May	4
Filterable serum	2	,,		None
Filterable serum followed by mosaic leaf juice after 10 days	8	,,	3rd May	1
Bacterial serum	2	,,		None
Bacterial serum followed by mosaic leaf juice after 10 days	8	,,	3rd May	1
Control mosaic leaf juice . .	3	,,	,,	1

These experiments showed that the sera were not able to confer immunity to the plants ; even the mosaic virus serum failed to show any prophylactic power. It is possible that the time allowed for the immunity to develop might be too short. Again the amount absorbed by the plant might be too little. These points await investigation before the question of immunity is finally settled.

CONCLUSIONS

A filter-passing cyclostage of bacteria isolated from mosaic-infected sugarcane leaves has been clearly demonstrated. Pleomorphic unstable bacteria which have only a very short cyclostage as bacteria but a long one as filter-passers is a new phenomenon which has been discovered.

The relation of this stable, filter-passing, invisible cyclostage to the sugarcane mosaic virus has been definitely brought home by the study of the serological reactions and a series of inoculation experiments in which the mosaic virus was neutralised by the anti-filterable forms serum ; and these experiments suggested that the mosaic virus was the stable cyclostage of a species of bacteria which have a very unstable existence in the bacterial stage. The bacterial stage was antigenically different from the other cyclostages and also from the mosaic virus.

The species of bacterium isolated differs from all known bacteria in its biochemical and morphological characters as would be expected from its cultural behaviours. The new conception of the etiological agent of the mosaic disease of sugarcane requires to be investigated by independent workers in this field, and with the idea of stimulating such research the experimental data here recorded are put forward in their present preliminary stage.

ACKNOWLEDGMENT

The writer is indebted to the Imperial Council of Agricultural Research under whose scheme for research on mosaic and other diseases of sugarcane the work has been carried out.

REFERENCES

Bewley, W. F. (1931). *Nature* **127**, 442.

Birkeland, J. M. (1934). *Bot. Gaz.* **95**, 419.

Bouquet, P. A. (1916). *J. Amer. Chem. Soc.* **38**, 2572.

Brehmer, W. (1931). Deuxeme Cong. Internat. Path. Comp. Tome Premiere Rapports, p. 355.

Brehmer, W. and Barner, J. (1930). Arb. Biol. Reichanst. f. Land-u. Forst. 18.

Desai, S. V. (1933). *Ind. J. Agric. Sci.* **3**, 626.

Eckerson, S. (1926). *Bot. Gaz.* **81**, 204.

Hadley, P. (1931). *J. Infect. Dis.* **48**, 1.

Melhus, J. (1922). Abstr. in *Phytopath.* **12**, 42.

Nelson, R. (1923). *Michigan Agric. Exp. Stn. Tech. Bull.* **58**.

———— (1932). *Michigan Agric. Exp. Stn. Tech. Bull.* **118**.

Smith, R. E. and Bouquet, P. A. (1915). *Phytopath.* **5**, 535.

Society of American Bacteriologists. (1928). Manual of Methods for Pure Culture Study of Bacteria, Supplement B, Serological Reactions. 2nd edition.

Swezy, O. and Severin, H. H. P. (1930). *Phytopath.* **20**, 169.

Takahashi, W. N. and Rawlins, T. E. (1932). *Proc. Soc. Exp. Biol. and Med.* **30**, 155.

STINKING ROT OF SUGARCANE

BY

S. V. DESAI, B.Sc., PhD., D.I.C.,

Sugarcane Mycologist, Pusa

(Received for publication on the 11th February 1935)

I. Occurrence

In July 1933 many sugarcane plants of Co. 300 and 313 were found to be wilting at Mushari Sugarcane Research Station. Most of the plants affected were found to be attacked by top shoot borers, but these insects very rarely kill the plants outright and the affected plants wilted in such a short space of time that other causes were looked for. The affected plants wilted completely within a fortnight and a peculiar fermenting smell was noticeable in fields having only a few plants so affected. The onset was sudden and all the leaves dried up though the weather conditions were very humid and favourable for the plants. The plants were examined and it was found that the disintergration and fermentation of tissues of the stem were taking place very rapidly from the top towards the bottom. The whole stem had become a mass of soft stinking pulp, and was found to be teeming with bacteria. The colour of the pulp was light grey and the offensive smell was very marked. In plants examined at this stage there was no sound tissue from which sections could be cut, but in plants showing incipient traces of the disease, the tissues a long way below the " dead heart " were found to be full of bacteria, mostly in intercellular spaces where the tissues had not disintegrated and in intracellular spaces as well in portions where fermentation was advanced. The vascular bundles were discoloured though these tissues did not disintegrate as did the other tissues. In advanced cases the leaves and the other dead tissues formed a favourable culture ground for many saprophytes, and the weather conditions favoured this secondary decomposition with the result that the leaves were black with moulds and other fungi. Insect maggots were also found in the fermented pulp, which mixed with the rain water and flowed all over the plant disseminating the offensive odour and attracting flies and other insects to the decomposing mass. The disease appeared to spread to adjoining stools which were quite healthy and without any top shoot borers ; such plants submitted suddenly to the rot and wilted within a short time.

II. Methods of isolation and cultures obtained

The bacteria responsible for this disease were isolated by plating from various affected tissues. The plating from pulped tissues invariably showed numerous saprophytic bacteria and yeasts, together with moulds and other saprophytic fungi. So many types of organisms existed in such tissues that it was difficult to find which one was responsible for the onset of the disease. The tissues below the rotted stem when minced up and plated showed a variety of bacteria and even here the causative organisms were hard to identify. The tissues still further below the rotted area were then tried, and from these it was found possible to isolate two types of bacteria, one white and other bluish on nutrient agar. The organisms grew well in the standard nutrient media and were obtained in pure culture by repeated plating in these media. No special medium was found to be necessary. The medium used contained :—

3·0 grams marmite
5·0 grams peptone
1000 c.c. water.
15 grams of shredded agar added to solidify the medium.

III. Pathogenicity

Pure cultures of these two types of bacteria were tried to find out their pathogenicity and reproduction of the typical disease by inoculations. At first the organisms were introduced into young plants by pin pricks, the organism being smeared over the surface and pricked in. Nothing beyond local lesion developed, which ultimately dried up in the case of the bluish type and in the case of the white type nothing except the wounds due to pin pricks were observed. Next the organisms were introduced into the plant by taking a plug out of the stem by a small cork borer. A culture was introduced into the hole, the plug was replaced and the stem tied up with tissue paper. In this case two plants out of six inoculated by the bluish culture showed disintegration of the tissues though not to a very great extent. Plants inoculated with the white organism remained healthy. Thus a slight pathogenicity of the bluish culture was brought out, while the white type was found to be unable to set up the disintegration. The infection took place only under excessively moist conditions.

The organisms were then introduced in borer holes of the borer-infected canes and a few marked as controls. It was then found that the bluish culture rapidly infected the tissues and set up the typical fermentation. Control plants remained unaffected and so did the plants infected in the borer holes by the white type. On trying to re-isolate the organism from the artificially infected plants it was found that the same two types of organisms were present and were readily isolated. It was at this stage suspected that the white type of organism though not parasitic was helpful to blue type which was pathogenic in setting up vigorous disintegration of the tissues.

A series of plants was therefore inoculated with white and blue types separately and together, and it was found that the mixed culture was markedly more efficient in setting up the fermentation and disintegration of the tissues.

The organisms were not strong parasites and when introduced by small surface wounds did not infect the plants; deep wounds and presence of dead tissues near about helped the infection and virulence developed after mass development of the organisms at the point of infection. These conditions were only fulfilled in the field when the organisms got entrance through borer holes or when mass culture of the organisms were introduced into healthy plants by contacts with the disintegrated tissues of neighbouring affected plants. The disintegrated tissues from decomposed lesions were found to be highly infective. The pathogenicity of both the organisms was also tested individually and collectively on hosts other than sugarcane. This was done because the bluish organisms resembled in some respects *Bact. marginale*, *B. aptatum* and *B. xanthochlorum* which are known to be pathogenic to solanaceous and other plants. Isolation of a similar organism from rotting potatoes has been reported by Hutchinson and Joshi [1915].

Potato, tomato, capsicum, brinjal and tobacco plants were infected by various methods and in varying conditions, but the organisms did not attack any of these plants. Bean plants were also inoculated but here too the organisms remained innocuous. Maize and *Euchlæna mexicana* were next inoculated but except for sugarcane the organisms were found to be non-pathogenic. The organisms were more infective in Co. 300 and Co. 313 than in Co. 213, though some amount of infection took place in other varieties of sugarcane tried. Later in the season, when the weather condition had become unfavourable, the infection did not spread rapidly unless the plant was covered up with a bell jar and kept in a warm place. Under these conditions the infection took its usual course.

Long cultivation on agar seemed to reduce the virulence of organisms for sugarcane.

When the artificial conditions of the test plants coincided with the natural condition of the bored plants the infection spread very rapidly and the typical smell and decomposition of the tissues took place.

IV. Biochemical reactions

Morphological observation and biochemical behaviour of the causative organisms are described below :—

These organisms were small rods 0·6-1·2 $\mu \times 1$ 2-2·2 μ in size without any spores or capsules, and were motile by single polar flagellum, which stained easily with any flagella stains.

Organisms were Gram negative and were easily discoloured by acid.

Ordinary Agar.—pH 6·5 at 30°C.

Colonies :—Greyish blue. Slight diffusion of greenish pigment in the agar surrounding the colony. Moist. Slightly raised.

Streak :—Greenish white, copious growth in water of condensation, diffusion of fluorescent pigment in the agar.

Gelatin :—Liquified within 48 hours. Crateriform, and fluorescent.

Nutrient broth.—Greenish yellow fluorescent turning deep green on shaking and assuming again the yellowish fluorescent appearance on standing. Clouding, slight ; pellicle at the top, no precipitate. The pigment decolourised by acid. Reaction of the medium neutral. Turning alkaline with evolution of ammonia.

Sugars, etc.—Glucose, saccharose, lactose, and glycerine fermented without gas. The reaction remains neutral. No fluorescence.

Milk.—Peptonized but not coagulated with zone formation. Top, bluish yellow, middle reddish blue and bottom unchanged in case of litmus milk. Production of yellowish pigment in ordinary milk with clear peptonization and slight precipitate at the bottom.

Potato.—Greenish yellow growth. Diffusion of greenish pigment in the potato tissues. Diastase present.

Uschinsky's solution.—Medium cloudy, slight pellicle.

Nitrate broth.—Nitrate not reduced to nitrite.

Indol.—Not produced. Hydrogen sulphide not formed.

Pectinase.—Present, dissolution of the middle lamella very marked. This property is responsible in the formation of pulpy mass in the rotting sugarcane as observed in the fields.

The characteristics of these organisms were compared with *B. aptatum*, *B. marginale* and *B. xanthochlorum* and the points of resemblance and difference are given in Table I.

TABLE I

Stinking rot	*B. apatatum*	*B. marginale*	*B. xanthochlorum*
1. Only one polar flagellum	Bipolar flagella	One to three polar flagella	One to three flagella
2. No spores	No spores	No spores	No spores
3. No capsules	No capsules	Capsules	?
4. Acrobe	Acrobe	Acrobe	Facultative acrobe
5. Gram negative	Gram negative	Gram negative	Gram negative
6. Not acid fast	Not acid fast	Not acid fast	Not acid fast
7. Agar colonies. Bluish, thin, smooth entire, flourescent, agar yellowish green, fluorescent.	Agar colonies smooth, flat, glistening, entire, internal fish scale markings. Agar green fluorescent.	Agar colonies cream becoming yellow, round smooth, thin, agar brilliant green.	Agar colonies yellow white to fluorescent
8. Milk peptonised but not coagulated, with zone formation. Top blue, middle reddish blue and bottom unchanged	Milk coagulated	Milk coagulated and turned green.	Coagulated and peptonised with green fluorescence
9. Gel. liquefied	Gel. liquefied	Gel. slowly liquefied	Gel. liquefied
10 Growth in sugars. No gas, neutral.	?	No gas, acidic. Dextrose	No gas, acidic. Dextrose and sucrose.
11. Ammonia produced	Ammonia produced	Ammonia produced	?
12. Nitrate not reduced	Nitrate not reduced	Nitrate reduced	Nitrate reduced
13. Diastatic action present.	No diastatic action	Diastatic action feeble	Diastatic action present
14. Fermi's sol:—Slight growth. Thin pellicle.	Thick pellicle and growth.	Heavy clouding and thick pellicle.	Some clouding
15. Conn's no growth	No growth	.....	.....
16. Indol not produced	Indol produced	No indol	Indol produced
17. Host (natural)	Host (natural	Host (natural)	Hosts
Sacharum officinarum	*Beta vulgaris*	*Lactuca sativa*	*Campanula ranunculus*
Non-pathogenic to *Capsicum annuum.*	*Tropaeolum majus Host (Artificial).*	.....	*Daucus carota*
Phaseolus vulgari, Solanum tuberosum.	*Capsicum annuum*	.....	*Lupinus nanus douglasi*
Nicotiana tabacum	*Lactuca sativa*	.....	*Nicotiana tabacum*
Lactuca sativa	*Phaseolus vulgaris*	.....	*Physalis alkekengi*
Zea Maize	*Solanum melongena*	.....	*Solanum tuberosum*
Euchlaena mexicana	.....	.....	*Vicia faba*

From this table it would be apparent that the organisms of sugarcane stinking rot belong to the group of *B. pyocyaneus* with a specialized parasitism developed against sugarcane and the name of *B. pyocyaneus saccharum* is suggested for the organism.

ACKNOWLEDGMENT

The writer is indebted to the Imperial Council of Agricultural Research under whose scheme for research on mosaic and other diseases of sugarcane the work has been carried out.

REFERENCE

Hutchinson, C. M. and Joshi, N. V. (1915). *Mem. Dept. Agric. Ind., Bact. Ser.* **1**, No. 5.

PARASITISM OF *SCLEROTIUM ORYZAE* CATT.

BY

B. B. MUNDKUR.

Assistant Mycologist, Imperial Institute of Agricultural Research, Pusa

(Received for publication on the 17th January 1935)

CONTENTS

I. Introduction

Investigations on *Sclerotium oryzae* Catt. associated with the rice plants (*Oryza sativa* L.) were started at Pusa in 1911-1912 followin upon the discovery of plants affected by this fungus in Bengal and Burma. Since those preliminary studies were conducted, the disease which this fungus was supposed to be causing did not attain the serious proportions which were first apprehended and interest in it seems to have waned in consequence. In 1930-1931 however a serious disease of rice which appeared in the Rajasahi and Dacca divisions of Bengal was associated with this fungus and investigations were therefore re-s arted in October 1931 to determine the factors which made this disease a severe epiphytotic and to find suitable measures for preventing and controlling the disease. It was also intended that a watch should be kept for types of rice plants that would resist the disease.

II. Review of literature

The sclerotial disease of rice was first described by Cattaneo [1879] from Italy where it was found to do a good deal of damage to the rice crop. He found that the culm of the affected plants was covered with a black discolouration near the soil level which later spread to all the leaf sheaths. Due to the pressure exerted by the parasite within the tissues, the epidermis of the plant burst and the plants withered and died. The name by which we know this fungus was given by that investigator.

At the time of distributing an exsiccatum of *S. oryzae*, Briosi and Cavara [1886] published a short description of the disease and the causal organism. The symptoms described by them laid stress on the black discolouration of the culm near the soil level and its ultimate rotting and they confirmed Cattaneo's observations in other respects.

The earliest mention of *S. oryzae* in India is by Butler [1913] who found it in association with the rice plants in Upper Burma. Some of the partially sterile plants did not have any insect attack but they were infested by the sclerotia of the fungus. In addition to this sterility, the plants showed the symptom of excessive tillering or the development of green sterile shoots from the basal nodes of the stock. The base of such plants was discoloured and was also invaded by fungal hyphae. The specimens were brought to Pusa where the associated fungus was identified as *S. oryzae*.

Very shortly afterwards Shaw [1913] published the results of an investigation carried out by him on this fungus. The chief symptom of the disease was stated by him to be tillering. The culm gradually turned yellow and died and if any panicle was borne, it was poorly filled and the grain was extremely light. The details of the cultural study conducted by him considerably supplemented the rather meagre description of the fungus given by Cattaneo [1879]. No infection experiments in pots or n the fields seem to have been conducted but the pathogenicity tests carried on in potato (Roux) tubes in which seedlings were grown and infected gave 70 to 80 per cent success provided the mycelium used for infecting the plants was young and vigorous.

A stem rot of rice ascribed to this fungus was described by Tisdale [1921] from Louisiana in the U. S. A. The affected plants had more or less entirely collapsed and the panicles were partially sterile. The stem nearer the ground, two or three nodes from the base, appeared darker and showed sclerotial infestation. The internode where the irrigation water stood was found to be completely destroyed, the epidermis alone remaining intact. Infection experiments conducted by him with the fungus were hundred per cent successful.

In Indo-China Vincens [1923] considered a sclerotial fungus to be the cause of the ' tiem ' disease of rice. The disease was characterised by a sparse growth of the plant, the stems of which became thin and leaves small, rigid, and pale.

He isolated from the base of dried leaves and from within the leaf sheaths a fungus which resembled *S. oryzae* in all respects.

According to Reyes [1929] the sclerotial disease of rice appeared to be the most destructive in the Philippines. The disease was detected in the field when the leaves began to turn yellow indiscriminately, followed by a stunting of the plants. Young leaves of the diseased plants were somewhat darker green and they tended to become stiff and erect. Three *Sclerotia* were isolated which were identified as three strains of *Sclerotium oryzae* and were designated as A, B, and C strains. Infection experiments in pots with the three strains gave 100 per cent success with a susceptible type of rice. Field tests gave from 20 to 93 per cent success.

According to Park and Bertus [1932] the sclerotial disease seemed to have been doing a good deal of damage to the rice crop in Ceylon. They state that it had been noted by Petch even as early as 1913. Park and Bertus found that the disease developed in a nursery in the seedling stage and one variety was so badly damaged that only a few of the plants survived. Examination of the affected plants invariably showed the presence of sclerotia which were identified as those of *S. oryzae*. Plants approaching maturity showed an unusual production of young shoots or tillers and the stalk gradually turned yellow and died. The infection experiments with seedlings gave on the whole successful results. Abnormal tillers were also noticed when infections were made on advanced plants, some affected plants even succumbed to the disease.

Symptoms of sclerotial disease as it occurs on the rice crop in Arkansas, Texas and Louisiana in the U. S. A., are described by Tullis and Cralley [1933]. The earliest manifestation of the disease was the presence of small black lesions on the outer leaf sheaths of the plants at the water line. The stem at this region showed considerable rotting as the spots enlarged and finally the tillers that were normally produced at the base were killed and the whole plants later succumbed to the disease.

From the rather detailed review of the symptoms of the disease of rice supposed to be caused by *Sclerotium oryzae* given above, it will be apparent that (1) the seedling disease due to this fungus, under natural conditions, has been recorded only in Ceylon, (2) that the phenomenon of excessive tillering has been associated with the disease by investigators at Pusa and Ceylon, and such a symptom has not been observed in Italy, Indo-China, the Philippines or the U. S. A., (3) that in these countries the most important symptom of the sclerotial disease is a stem-rot which leads to a yellowing and drooping of the plants and finally to their death and that these symptoms have not been recorded in India. In all cases however the sclerotia of *S. oryzae* were unmistakably present in the culms. There are thus considerable differences in the symptom-picture of the

disease as described, for in India the acute rot of the stem has not been observed either by previous investigators or by the writer while the symptoms noted here have not been found elsewhere.

III. *Sclerotium* spp. included in the investigations

Cultures of *Sclerotium oryzae* and other *Sclerotium* spp. for the study were assembled from several sources. In many instances straw bearing the sclerotia was secured from different geographic locations in India from which the fungus was isolated. Many of the cultures were kindly sent by pathologists in other countries.

In making the isolations, pieces of straw bearing the sclerotia were washed with mercuric bichloride solution (1 : 1000) and then with sterile water. These pieces were transferred to a sterile Petri dish where the sclerotia were aseptically teased out and transferred to oat agar (five per cent Quaker oats and one and one half per cent Bacto agar). Pure cultures of *S. oryzae* and other *Sclerotium* spp. were thus obtained.

As soon as the cultures were isolated or were received from abroad, single sclerotia were picked on a transfer needle and planted on potato-glucose agar (one litre of clear infusion from 200 grams of peeled and cut potatoes + 2 per cent glucose + 2 per cent Bacto agar). The cultures of *S. oryzae* produced after about 10 days a coral pink colour (Ridgway : ' Colour Standards and Nomenclature ') on this culture medium, which later became jasper red. This characteristic helped in sorting out the cultures of *S. oryzae* for none of the other sclerotia-forming fungi that were encountered in this study had this property. Production of various shades of pink by this fungus has been noted by Shaw [1913] and Park and Bertus [1932] on some substrates including maize-meal agar. When that agar was made according to the formula of Shear and Wood the cultures did not develop the pink colour in the writer's studies. The colour on the contrary was olivaceous to greenish mixed with slaty grey. The colour production was noted when the cultures of *S. oryzae* were grown on the following other media : Lima bean agar, Brown's synthetic agar and oat agar. Deepest colour production was however on the potato-glucose agar. It was noted that the intensity of the colour produced depended on the variety of potato used in making the medium and on the strain of *S. oryzae*. For example the intensity of colour produced on a medium made from potatoes sold at Pusa as ' Hill potatoes ' was not so intense as on that made from local (' Dehati ') potatoes. Likewise isolate Nos. 1, 3 and 4, (Table I) produced more intense colour than the rest of the strains.

When the fungus was grown in flasks on potato-glucose agar, the progressive change in the colour of the medium could be watched. Some isolates changed the colour quickly while the others were rather slow in doing so. Colour production did not seem to depend on light for inoculated flasks placed in a dark

chamber developed the colour. If the flasks were left for eight to ten weeks, the colour slowly faded and after sometime a faint indication of pink could be seen. When the isolates were grown on potato-glucose broth (the above ingredients without the agar) in flasks, colour was produced with equal intensity and when the liquid was filtered, the filtrates were scarlet. The description of the cultures included in the study follows :—

TABLE I

Cultures of Sclerotium *spp., used in the comparative study*

Serial No.	Description
1	Isolated from straw obtained from Dacca. October 1931. *S. oryzae.*
2	Sent by M. Park, Ceylon, February 1932. Chromogenic strain of *S. oryzae.*
3	Sent by E. C. Tullis, U. S. A. Originated in a single ascospore from *Leptosphaeria salvinii* Catt. April 1932. *S. oryzae.*
4	Sent by U. Thet Su, Burma. May 1932. *S. oryzae.*
5	Sent by H. Chowdhury, Punjab, October 1932. *S. oryzae.*
6	Sent by T. Nakata as *S. oryzae.* September 1932.
7	Isolated from straw, Anakapalle. *S. oryzae.*
8	Isolated from straw from Mymensingh. *Sclerotium* sp. October 1931.
9	Sent by M. Park, Ceylon (? *S. oryzae,* non-chromogenic strain*).
10	Sent by U. Thet Su, Burma as *S. oryzae,* large size.
11	Sent by T. Nakata, Japan, as *S. sphaeroides.* May 1932.
12	Sent by T. Hemmi, Japan, as *S. oryzae sativae* Sawada, May 1932.

(*See appendix)

In addition to the above, *S. oryzae* has been isolated from rice straw received from Jorhat in Assam, Kalashakaku and Karnal in the Punjab, Karjat in the Bombay Presidency and from Kashmir. *Sclerotium* resembling accession No. 8 has also been isolated from rice straw from Kalashakaku, Karnal, Chinsurah, Motihari and Maruteru. Two more sclerotia-forming fungi were isolated from

some of the rice straw, one of which resembled a culture sent by Dr. T. Nakata under the name *Sclerotium microsphaeroides* Nakata, while the other one is of the *Rhizoctonia* type. Studies with these are not yet complete and they have not been included in this report. None of the Indian isolations resembled the fungus sent by Dr. T. Hemmi as *Sclerotium oryzae-sativae* Sawada and as it did not seem to occur here, the culture and the sub-culture were killed in order not to introduce a new fungous parasite into the country.

IV. Morphological studies

Extensive experiments to determine the influence of physico-chemical factors on *S. oryzae* were planned but when the pathogenicity tests of 1932 indicated that the fungus under Pusa conditions did not produce disease, laboratory work was considerably slackened and it was stopped completely when the results of 1933 experiments confirmed the previous year's negative results. Enough however was done to determine a few growth characteristics and morphological features so that it is possible to define the *Sclerotium* spp., associated with the rice straw in India.

S. oryzae Catt.—This *Sclerotium* seemed to have a preference for those media which contained an infusion from cereals or vegetables. On Brown's synthetic agar, which contains starch, growth was fair but on completely synthetic media like the Czapek's, Richards' and Coons' growth was extremely poor and sclerotial formation scanty. Production of a coral pink to jasper red colour by this fungus on certain substrates has already been noticed.

A white fluffy growth began to appear on potato-glucose agar the third day after making a transfer and sclerotia appeared on the ninth day together with smoky lobed appressoria. The mycelium became olivaceous to black at the edge of the medium in the test tube but not in all the strains, especially isolate No. 3. Some isolates (*viz.* 3 and 7) formed more aerial than submerged mycelium, which was of a pale drab gray to smoky gray colour. A thick layer of sclerotia was formed just beneath this aerial growth.

The young mycelium from a potato-glucose agar tube was hyaline but as it aged it developed a pale amaranth pink colour. Branching was profuse and at the base of the branch there was a slight constriction and a basal septum and this faintly resembled the mode of branching in *Rhizoctonia solani* Kuhn. The older mycelium was highly vacuolated and chlamydospore-like structures mentioned by Shaw [1913] abounded. It was observed that the mycelium from onion agar where the substratum was not discoloured, except at the edges, where it turned black, was sub-hyaline. The mycelium was from 3 to 5 μ broad and the length of the cells varied from 40 to 225 μ.

The cultures were grown on oat agar for obtaining an abundance of sclerotia for the purpose of measuring their diameters. The cultures were a month (30 days) old at the time of measurement. The sclerotia were picked from any random

place, mounted in lacto-phenol (one part each of lactic acid, phenol, glycerine and water by weight) mounting medium and two diameters at right angles to each other were measured and were then averaged. By accident 210 sclerotia were measured at the first instance and the same number was measured in the other cases likewise for the sake of uniformity. There is thus not any special virtue about that number.

The frequency distributions of the sclerotial measurements, the range, the mean and its standard error and the coefficient of variability are given in Table II.

TABLE II

Frequency distributions and biometric constants in microns of diameters of seven isolates of Sclerotium oryzae

Isolate No.	Classes																		Biometric constants.	
	156	182	208	234	261	287	313	339	365	391	417	443	469	495	522	548	574	600	*Mean	*Coefficient of variability per cent
1	...	5	14	56	57	46	27	10	4	1	...	...	...	...	...	...	...	...	266±2·63	14·3±0 71
2	...	...	11	34	47	47	43	11	7	3	4	3	1	...	...	...	...	...	286±3·32	16 8±0 84
3	3	17	31	41	69	33	15	1	...	...	...	...	...	...	...	...	...	...	242±2·48	14·5±0·72
4	...	4	35	61	79	24	6	...	1	...	...	...	...	...	...	...	...	...	284±1·88	10·9±0·54
5	...	...	2	7	7	8	18	13	25	20	29	20	21	13	8	10	8	1	405±5·97	21·3±1·08
6	...	...	...	2	9	20	37	40	50	36	12	4	...	...	...	...	...	...	347±2·46	10·3±0 51
7	...	...	6	17	29	36	46	34	19	14	8	1	...	...	...	...	...	...	310±3·43	16·0±0·80

* In this and other tables, standard errors are always given.

In the data recorded in Table II, it will be noted that the range of the diameters of the sclerotia of the different cultures varied from 156 to 600 μ and the mean diameters from 242 to 405 μ. The isolate with the largest mean diameter showed rather high variability in size so that it would have been better had a larger number been measured. The measurements given are in general accordance with those recorded by Shaw [1913] and Tisdale [1921]. The mean diameter of the Ceylon strain is 286 ± 3·32 μ and the diameter of this same *Sclerotium* according to Park and Bertus [1932] is 374·5 + 4·3 μ. This discrepancy can be explained by the fact that they did not secure their sclerotia at random but selected them from one half inch from the point of inoculation in a maize agar tube. It is possible therefore that this is the cause of the discrepancy.

Briosi and Cavara [1886] found the sclerotial diameters to be 150-180 μ but Ashby states (in a personal communication) that he measured 16 sclerotia from their exsiccatum which gave a range of 225-330 μ with a mean diameter

of 270 μ. The writer measured ten sclerotia from their exsiccatum that is in the Pusa herbarium and the range obtained was 208-339 μ with a mean diameter of 262 μ.

It was of interest to determine whether the diameters of sclerotia of culture No. 3 (sent by Dr. Tullis) which had its origin in a single ascospore (in its *Leptosphaeria* stage) showed any consistency in size in view of the variation shown by the sclerotial bodies. The fungus was grown on oat agar for thirty days, a sub-culture for the second clonal generation made, and then two hundred and ten sclerotia were measured. The second clonal generation was also grown for thirty days, a sub-culture for the third clonal generation made, and the sclerotia were measured. The third generation sclerotia were also measured after thirty days. The frequency distributions and other biometric constants will be found in Table III.

TABLE III

Frequency distributions of sclerotial diameters of three clonal generations

Progeny	Classes in microns								Biometric constants	
	156	182	208	234	261	287	313	339	Mean ± S. E.	C. V. ±S.E.
3_1	3	17	31	41	69	33	15	1	242 ± 2·48	14.5 ± 0·72
3_2	1	16	23	39	76	26	17	12	258 ± 2·57	14.4 ± 0·71
3_3	2	13	30	47	66	36	14	2	251 ± 2·43	14.0 ± 0·69

These frequency distributions were then compared using the Pearsonian method suggested by the writer [Mundkur, 1934] cell by cell with a view to see whether they could be considered as having descended from the same parent. The value of χ^2 and P (Fisher's table) are given in Table IV.

TABLE IV

Comparison of sclerotial diameters of three clonal generations of S. oryzae, *culture No. 3*

Generations compared	χ^2	P(between)
3_1 and 3_2	11.8645	0.10 to 0.20
3_1 and 3_3	1.6082	0.98 to 0.95
3_2 and 3_3	12.0625	0.10 to 0.20

From the data recorded in Table IV it will be observed that the values of P are sufficiently high. According to Fisher [1932] if P is between 0·1 to 0·9 there is no reason to suspect that the differences in mean diameters are such as to rule out the hypothesis that the sclerotia belong to the same general population or a common parent. It is only when the value of P is 0·02 or below that a strong indication points to their not belonging to such a population. As all the values of P in the table are within the non-significant range, it can be concluded that the mean differences in the diameters of the sclerotia are not such as to preclude the possibility of their belonging to the same parent which we know they do in terms of the experiment.

Tullis [1933] has reported that *Helminthosporium sigmoideum* Cavara is the conidial stage and *Leptosphaeria salvinii* Catt., the ascigerous stage of *S. oryzae*. He obtained the former stage in culture media as well as on affected plants while the latter stage (ascigerous) was obtained only in the affected leaf sheaths and culms. This conidial stage was also obtained by him in cultures of *S. oryzae* sent by the writer from Pusa. No such conidial stage was observed in culture media at Pusa but when the agar from the culture tubes bearing the mycelium was asceptically taken, placed on a sterile watch glass and incubated in a sterile moist chamber in diffuse light, conidial production has been obtained. The Indian and Burmese strains of the fungus belong therefore to *H. sigmoideum* in their conidial stage and therefore to *L. salvinii*, the ascomycetous stage, by analogy.

Sclerotium sp.—Isolates included under this heading comprised accession Nos. 8, 9, 10 and 11. Park and Bertus [1929]* considered this as the non-chromogenic strain of *S. oryzae* but their detailed account which seems to have been sent up for publication* (*vide Adm. Report, Ceylon Dept. of Agric.* 1931, p. D 111) is not however yet available.

The culture sent by Dr. Tanaka was labelled '*S. sphaeroides* Nakata, formerly called Sakurai's *Sclerotium* No. 2.' A search in literature to find out where the fungus may have been described by him proved fruitless, neither could it be ascertained from the author himself.

Cultural studies revealed that these four isolates belonged to one fungous species which differed from *S. oryzae* in several respects. Grown on oat or potato-glucose agar media, there was not any production of colour in the substrates which retained their original tint. The fungus did not seem to have any discrimination against synthetic media for it grew well on all the common media, vegetable, cereal or synthetic. It was noted that sclerotial formation started within six days of planting a sclerotium of the fungus on a nutrient medium. After some time aerial growth appeared to be rather scanty, for all the aerial hyphae had by this time been turned into sclerotia of which there was an abundance. Hyphae within the substratum were however plentiful.

* See appendix.

The young mycelium from a potato-glucose agar tube did not show much branching but the older mycelium was profusely branched. It was hyaline and there was not any development of colour within the hyphae, however old. Chlamydospore-like structures and the appressoria were absent. The constriction at the base of a branch was more of the *Rhizoctonia* type. Septation was close, the length of cells being 25 to 80 μ and the breadth from 3 to 5·5 μ. Clamp connections were rather rare.

The sclerotia appeared smooth but they really had a rough surface due to slight protrusions. The sclerotia were spherical, sub-spherical or oval, and sometimes three to four sclerotia were fused. To begin with they were whitish but later they turned brown and finally to fuscous black. Hardly any of them were submerged within the substratum. Their diameters ranged from 217 to 713 μ. Two hundred and ten sclerotia of each culture were measured and their mean diameters and other biometric constants are recorded in Table V.

TABLE V

Biometric constants of sclerotia of Sclerotium sp.

Isolate No.	Range	Mean ± S. E.	Coefficient of variability ± S.E.
	μ	μ	Per cent
8	269 — 713	443 ± 5.16	16.8 ± 0.84
9	269 — 713	465 ± 5.95	18.6 ± 0.93
10	269 — 713	446 ± 4.92	15.9 ± 0.79
11	217 — 609	442 ± 5.93	19.4 ± 0.98

The data recorded in Table V shew that there is not much variation in the mean diameters of the sclerotia, excepting that the culture from Ceylon seemed to have rather large sclerotia.

The question of nomenclature of these isolates now remains to be settled. This fungus (all the four isolates) differs from *S. oryzae* in the inability to produce pink colour on certain substrates, in the ability to grow profusely on synthetic media, in the mycelium being hyaline, in the absence of smoky lobed appressoria and the chlamydospore-like structures, and in the size of sclerotia which are decidedly larger. It has not formed the *H. sigmoideum* stage even though several methods to induce the stage were tried. It can by no means be considered therefore as the non-ehromogenic stage of *S. oryzae*.

Nakata also seems inclined to consider this as a distinct fungus for he calls it *S. sphaeroides* but it cannot be designated by that name as this binomial has already been used by Massa [1912] to describe a sclerotial fungus on *Lychnis dioica* L. in Italy.

But the branching of the hyphae in this fungus is very typical of *Rhizoctonia*, for there is the typical constriction at the base of the branch and also the basal septum. The sclerotial surface is not smooth but rough as in the case of *Rhizoctonia* and the fungus has therefore to be placed in that genus. Ashby to whom a culture was sent for the purpose of identification compared it with a culture of *R. microsclerotia* deposited at the Centraalbureau voor Schimmelcultures, Baarn, Holland by Dr. T. Matsumoto and found that it differed from it in several respects. But Ashby states (in a personal communication) that the culture sent from here tallied in several major respects with the published description of *R. microsclerotia* given by Matz [1917, 1921]. Even though Matz mentions a corticial stage which has not been observed in the culture here, the writer is disposed to agree with Ashby. As an authentic culture of Matz's strain is not apparently available, cultures were submitted to Dr. J. Matz for his judgement in the matter and he wrote :

> "Two of the tubes contained unmistakably the fungus *Rhizoctonia microsclerotia* and were inscribed with the number 59. I am sorry I am unable to send you cultures of my *Rhizoctonia microsclerotia* or *R. alba*."

(The number 59 mentioned above refers to number 8 used in this publication).

The Indian, the Burmese, the Ceylonese and the Japanese cultures are therefore identified as those of *Rhizoctonia microsclerotia*. A culture of a *Sclerotium* isolated from the Philippines rice straw was received from Dr. C. J. Humphrey, and it was identical with the cultures of *R. microsclerotia*. A culture of isolation No. 8 has been deposited at the Centraalbureau at Baarn as it has been authenticated by Dr. Matz, Mr. Ashby and the writer.

V. Infection experiments

Infection experiments were started in the rice season of 1932 both in pots and fields. The pots were usually of glazed earthenware and were filled with soil from the rice fields. An adequate number of them were steam-sterilised under pressure for two hours. Four plants were usually sown in each pot, unless otherwise stated.

There were three plots for conducting the field experiments, each 26 × 18 feet, lying side by side, in a north to south direction. In 1932 it was noted that these plots were badly infested by termites. The soil to a depth of a foot was therefore removed and replaced with soil from a good rice field in April 1933. The centre plot served as a check plot. The other plots and the pots were infested

with cultures of *S. oryzae* in the fourth week of April each year. These cultures were grown on large quantities of a sterilised mixture of paddy and straw in 1500-c.c. flasks. The fungus was allowed to grow in the flask for six weeks during which time it formed profuse sclerotia. Contents of six such flasks were added to each of the two plots which were ploughed soon after and irrigated. The pots were likewise infested with large quantities of the fungus and irrigated, and water was allowed to stagnate both in the pots and the fields. The plots were re-infested in 1933 and 1934 and during these years additional infestation was also done after the crop was eleven weeks old. In 1934 further infestation was done by adding straw bearing the sclerotia to the soil in the two infested plots.

At the time of sowing, the seed was mixed with the sclerotia in 1932 and 1933 but not in 1934. The plots were sown with seedlings raised in the nurseries. They were dipped at the time of sowing in a suspension of sclerotia. It will therefore be noted what great care had been taken to see that infestation of the soil by the fungus was particularly heavy. The infectivity of the soil was therefore all that could be desired provided of course that the fungus was pathogenic.

Experiments in 1932.—The seed for these experiments was obtained from the Dacca Agricultural Farm where the disease had appeared in an epidemic form in 1930-1931. The seed was designated *Indrasail* and was supposed to be particularly susceptible to the disease. There were 24 pots containing sterilised soil and the rest had ordinary soil. The results are tabulated in Table VI.

TABLE VI

Infection experiments in 1932 with cultures of Sclerotium oryzae

Date of sowing	No. of pots	Total plants	Culture number	Number attacked
June 10 . .	5	20	1	None
June 10 . .	4	16	check	None
June 10 . .	4	16	2	None
June 10 . .	5	20	3	None
June 10 . .	6	24	4	None
June 28 . .	4	16	1	None
June 28 . .	6	24	3	None
July 19 . .	5	20	1	None
September 21 .	6	24	1	None

The tests conducted in July and September were done in ordinary flower pots in unsterilised soil. None of the plants showed any symptoms of the disease at any time. There were not any lesions or any excessive tillering and the panicles were normally filled with grain. In the pots sown in June and July the fungus was not only incorporated with the soil and given with the water on two occasions (July 13th and October 10th) but agar bearing the mycelium was placed within the leaf sheaths. There were no symptoms of the disease even then. The pots sown in July and September flowered rather late, that is in November, but the experiment was terminated at the same time as the June pots and it could not therefore be ascertained whether they bore well-filled panicles or not. After harvest the straw was stored in a dry place and when examined a week later it showed the presence of a large number of sclerotia.

Field tests in 1932.—The seed was sown in beds on the 3rd of June 1932 and transplanted on the 16th of July 1932. The crop was carefully watched. In July and later, several plants suddenly dried up and died. These were picked and examined when it was found that they had all succumbed to termite attack. Such deaths took place even in the check plot. The crop suffered from leaf spots due to *Helminthosporium oryzae* Breda de Hann, but of the symptoms of the sclerotial disease, there were none. There was no excessive tillering or stem rot and the plants bore well filled panicles. After harvest in November the straw was examined for the sclerotia. Several of the plants bore these in the leaf sheaths but they became appreciably greater in numbers after about a week when the straw had partially dried.

Experiments in 1933.—The tests during this year were carried out in the same manner as in the previous year, except that the quantity of inoculum in the plots and pots was more than last year. Furthermore three more isolates collected during the previous year were also tested for their pathogenicity in the pot experiments. The seed for the experiments was secured from the Economic Botanist, Dacca and was designated Chinsurah No. 1 by him.

Pot experiments.—The results of the pot experiments are recorded in Table VII. The soil in the pots this year was not sterilised.

TABLE VII

Infection experiments in pots with Sclerotium spp. *in 1933*

Date of sowing	No. of pots	No. of plants	Culture used	Number attacked
3rd June 1933 . . .	5	20	1	None
3rd June 1933 . . .	6	24	3	None
3rd June 1933 . . .	6	24	4	None
3rd June 1933 . . .	6	24	5	None
7th June 1933 . . .	3	12	6	None
7th June 1933 . . .	6	24	7	None
7th June 1933 . . .	4	16	8	None
7th June 1933 . . .	5	20	11	None

As glazed pots were not available for all the tests, ordinary flower pots were used for isolates Nos. 6, 7, 8 and 11.

None of the plants showed any of the disease symptoms caused by *S. oryzae*. There was no abnormal tillering and the plants were not sterile. In ten ordinary pots seed had been sown but the soil was not infested with the fungus. These also remained healthy. It was noted that the plants did not bear well and this feature was manifest in the control pots also. On the whole the Dacca seed did not seem to be well adapted for growth under Pusa conditions.

Several trials were made in June-July 1933 in small four inch pots to see if the disease could be produced in the seedlings. There were twenty such pots and to each one of them large quantities of the fungus were added. Six seeds were sown in each pot and the seedlings were kept under observation for about five weeks, but there were no signs of any seedling disease. The pots were then abandoned.

As Shaw [1913] and other investigators obtained successful results when they infected the plants growing in test tubes, similar tests were done in the month of July, 1933. The technique followed was the same as described by the writer [Mundkur, 1926] and as a liquid culture medium Shive's R_5C_2 solution was used. In addition to large test tubes, one inch broad and nine inches tall, seedlings, were raised in Roux tubes. In all there were twenty-eight large test tubes and thirty-six Roux tubes. The seed used in these studies was the same Dacca seed as used in the pot experiments and it was, prior to use, surface-sterilised for three hours in a solution of calcium hypochlorite (bleaching powder) as recommended by Duggar

and Davis [1919]. Two seeds per test tube were placed on 6th July 1933 and these test tubes were kept in a warm place. The seed emerged within four days. On the tenth day when the seedlings were about two inches high, they were infected with sclerotia and vigorously growing mycelium from isolates, No. 1, 2, 3, 6, 7, 8, 9, 10 and 11. There were six tubes for each culture and ten served as controls.

The tubes were placed first in diffuse light and later they were transferred to strong light. On the 14th day, that is four days after infection, some of the plants showed lesions and this symptom became general two days later in all the infected tubes. The mycelium was also seen to be growing well. Three weeks after the sowing of the seed in the tubes, the plants began to die and most of them were dead within the twenty-fifth day. The plants in the controls showed no such symptoms and remained very healthy.

The infected tubes were abandoned and on the twenty-seventh day five of the control tubes were infected with isolate No. 1. The plants completely succumbed to the disease within twelve days. The rest of the controls remained healthy.

Field experiments in 1933.—These were done on the same lines as in the previous year except that more inoculum was added to the soil. The plots were sown with four week old seedlings on 12th July 1933 and the growth of the crop in all the three plots was good. The crop suffered from leaf spots due to *Helminthosporium oryzae* and to a slight extent, *Piricularia oryzae* Br. and Cav. The plots were harvested in November. There was no excessive tillering, sterility or any stem rot. The infected plots yielded 2 lbs. 6 ozs. and 2 lbs. 13 ozs. each and the check plot yielded 2 lbs. 9 ozs. seed. The yield on the whole was not good.

The leaf sheaths of the growing plants from the infected plots were examined from time to time to see if the fungus was present. In October some leaf sheaths from the infested plots showed the presence of the fungus within the tissues and the appressoria mentioned by Tullis and Cralley [1933] were noticed together with a few sclerotia. After the straw was harvested, a large number of sclerotia were noticed in the sheaths. No such fungous infestation was observed in the culms or leaf sheaths of plants in the check plot and, when the straw was again examined a month after harvest, there were no sclerotia.

Experiments in 1934.—The infection experiments were repeated on the same lines as in previous year with this exception that the seed sown in pots was of Type 31, a type obtained from the Botanical Section at this Institute. Instead of seed, seedlings were sown in the pots, soil in which was not, however, sterilised. The isolates used for infestation of the soil in the pots were No. 1, 3 and 7. Seedlings of Shoemed rice, which is a very susceptible type in Arkansas and Louisiana to the stem-rot disease and which had been kindly sent by Dr. E. C. Tullis, were transplanted in the plots on July 3, 1934.

Pot experiments in 1934.—The results of the pot test are given in Table VIII.

TABLE VIII

Infection experiments in pots with Sclerotium oryzae

Date of sowing	No. of pots	No. of plants	Culture used	Number diseased
6th July 1934 . . .	16	64	No. 1.	*Nil*
6th July 1934 . . .	14	56	No. 3	*Nil*
6th July 1934 . . .	10	40	No. 7	*Nil*
6th July 1934 . . .	12	48	control	*Nil*

It will be noted that as in previous years, no disease was recorded in the pots.

Field experiments in 1934.—On the 18th of August, additional quantities of sclerotia and mycelium growing in giant cultures were added to the soil in the two plots. In September and October, plants were up-rooted and examined for sclerotia but none were found to be infested. Several plants in the infected and the check plot died in October due to termite attack. The straw was again and again examined with negative result. In late October sclerotia were noticed in the leaf sheaths of the plants from the infected plot. The presence of the sclerotia did not mean any disease, however, for none of the symptoms was seen at any time.

VI. ASSOCIATION OF *S. ORYZAE* WITH THE RICE CROP IN OTHER PARTS OF INDIA

The negative results of pot and field experiments led to the diseased plants being looked for in the fields at Pusa and elsewhere. The Economic Botanist in charge of rice investigations at Dacca (Bengal) wrote that the rice disease at that place had been definitely traced to borers which attacked and killed the plants. In the straw of such plants the sclerotia of the fungus invariably appeared. He was of the opinion that *S. oryzae* may not be the initial cause of the disease and that if it did play any role at all, it was of a secondary character.

The Government Mycologist at Coimbatore (Madras Presidency) stated that *S. oryzae* had been isolated on several occasions from rice straw but that it may be, if at all, a weak parasite and caused no serious damage to the crop in that presidency.

The Associate Professor of Botany, Lyallpur, wrote that in the Punjab the disease was doing a good deal of damage. The Director of Agriculture, Kashmir, reported in 1933 that the rice crop in the State showed unusual sterility and that he considered that it may be due to some fungous disease. The Rice Specialist,

Bihar and Orissa, who has extensively toured in the rice tracts of the Province and who has the crop under close observation at Sabour, stated that no sclerotial disease as described by Butler or Shaw was noticed by him anywhere in the Province, nor had he seen any stem-rot.

A watch for this disease has been kept in the rice fields at Pusa and the neighbourhood. The disease has not been observed during the last three seasons though the harvested rice straw has shown the presence of the fungus in some cases.

In 1932 Dr. M. Mitra visited the Rice Breeding Station at Karjat, near Bombay. The Crop Botanist who has this crop under close observation had not observed any stem-rot disease that could be traced to fungi nor had he noticed any unusual sterility or tillering in his plots. But rice stubble left in his plots showed infestation by the fungus as also the rice straw from the same fields. It was manifest that the fungus was there in the soil and may have even attacked living plants but it evidently did not produce any disease.

In 1933 the writer visited Anakapalle, Samalkota, Maruteru, Coimbatore and Pattambi, all agricultural experiment stations in the Madras Presidency. The Superintendents in charge of these stations stated that there was no sclerotial disease of rice at these places and that the crop every year was normal. At Anakapalle the straw and stubble from plots that had just been harvested showed the presence of the fungus. The panicles were heavy with grain but the leaf sheaths were full of sclerotia. Evidently the crop had not suffered from sterility even though the fungus was present.

At Maruteru the writer was shown several plants which showed excessive tillering. Seven such plants were uprooted and dried. Three plants showed infestation by the fungus when examined at Pusa and four did not. At Maruteru itself several such plants failed to show any infestation by sclerotial fungi.

Such plants were also a feature at Pattambi. Four plants were brought to Pusa and examined but there was no trace of any sclerotial fungi. It further appeared that this fungus was absent at this farm, for, several samples of straw from this station, that were brought over for examination, failed to show any sclerotial infection.

At the Coimbatore Rice Experiment Station, sclerotial infestation of standing plants as well as harvested plants was noticed. There was not any sterility or excessive and abnormal tillering. The investigators at this place had not observed the stem-rot symptoms either. The only disease that was causing concern was the foot-rot due to *Fusarium moniliforme* Sheld, and this had been controlled to a large extent by seed treatment.

On the Karnal Farm in the Punjab the writer examined several healthy plants with heavily filled panicles. Some of these showed sclerotia in the leaf sheaths but the plants could under no circumstances be considered as being diseased. No where were any plants showing the stem-rot symptoms as described by Reyes or Tullis and Cralley ever noted.

No opportunity to visit Western and Northern Punjab and Kashmir during the rice season presented itself but even though the specimens of rice plants sent by the Director of Agriculture, Kashmir, showed sterility, only two plants, out of seven plants received, showed infestation by the sclerotia. The others were free from it. The culture of *Sclerotium oryzae* received from the Punjab gave negative results in the experimental tests already cited in Table VII.

VII. Discussion of results

Data have been presented in the foregoing pages which seriously question the capacity of *S. oryzae* to produce disease in rice plants under conditions obtaining in some parts of India. The isolates used in the investigation were not only of Indian origin but several of them were received from pathologists abroad at whose hands they had proved to be pathogenic to rice plants, producing the well known stem-rot disease. On plants growing in water cultures in large size test tubes, all these cultures were equally parasitic and brought about a seedling disease.

In pot experiments extending over three years and comprising over 100 pots heavily infested with sclerotia of *S. oryzae*, this fungus has shown itself to be incapable of bringing about the disease at Pusa. The straw when harvested has shown the presence of the sclerotia in the tissues of the culms and the leaf sheaths, leaving no doubt regarding the presence of the infective material in the soil.

The field experiments conducted during the past three years have further confirmed the above observations. In two plots that were heavily infested with the fungus initially and again at intervals, no disease developed at any time. There was not any abnormal tillering or any marked sterility which was not a feature of the check plot plants as well. None of the plants showed any stem-rot and the few deaths that took place in the fungus-infested as well as check plots were definitely found to be due to termite attack.

The yield of the plots was not less than that of the check plot. In 1933 living plants at various stages of growth were occasionally examined to see if the fungus could be seen within the tissues. In late October, when the plants were about five months old it was noticed that in the tissues of leaf sheaths fungal hyphae which were sub-hyaline to brown in colour were present intercellularly and peculiar appressoria-like structures were also present. Just before harvest even sclerotia were noticed within the tissues. They appreciably increased when the plants were harvested and the straw was dried. There was however no disease and the plants bore well filled panicles.

It was apparent that the fungus was able to effect entry within the host plants but it evidently could not make enough progress so as to kill the plants or produce other disease symptoms as a result of this infection. It was noted that at the time the fungal hyphae were observed in the tissues, many of the older bottom leaves had dried up and possibly the fungus enters through such leaves. Such dead

leaves were a feature of the plants in the check plots as well but the tissues of leaf sheaths there did not show the presence of the fungus nor were there any sclerotia seen in the straw of this plot after harvest.

Field observations made at various places furnished further confirmatory evidence to show that while the fungus was able to infest the plants at a rather late stage of their growth, it was not able to bring about the disease. At many agricultural stations where the rice crop was under study the investigators were unable to detect abnormal tillering, sterility or stem-rot but in their plots left over stubble invariably showed the presence of the sclerotia which were also found in the harvested straw. These sclerotia were either of *S. oryzae* or *R. microsclerotia*.

Standing plants in the fields bore well filled panicles but at the base of the leaf sheaths sclerotia were invariably noticed. Sterile panicles also showed such infection. Presence of sclerotia was not invariably an indication of sterility. Rice straw from Pattambi was free from sclerotial infection and possibly this fungus does not occur there but, all the same, plants with abnormal tillers and with sterile panicles were present in the fields there.

Jones [1930] has shown that some types of rice plants show two to ten per cent sterility. This sterility increases when such types are hybridised, for in the hybrids between these the percentage of sterility in the F_2 has been as much as 48. Jones has ascribed this sterility to incompatibility in the chromosome mechanism. It is possible that to some extent the sterility seen in the rice plants may have been due to such a cause.

Taking all the above facts into consideration, it is permissible to ask whether *S. oryzae* is at all pathogenic to rice and if it is, how it can occur in association with its host without causing the disease which it is known to bring about elsewhere.

That it is a parasite of a rather virulent nature in Italy, Indo-China, the Philippine Islands and the U. S. A., is clear from the reports of pathologists in those countries. It is also manifest from the investigations of Park and Bertus [1932] that the fungus causes a seedling disease in the crop and possibly abnormal tillering in mature plants in Ceylon. But in India different conditions of environment prevail and even the nature of the soil is different. Though the fungus evidently attacks the plants because its presence in living plants in the basal tissues has been noted, it does not cause any appreciable effect on the growth of the host or injure it by bringing about stem-rot. That such a phenomenon is possible is apparent from several instances that can be cited. *Endothia parasitica* (Murr.) Anderson et Anderson is not a parasite of any consequence to chestnuts in Japan where the fungus is endemic. But this fungus has wiped out the chestnut trees on the Atlantic Seaboard of the United States of America. Similarly in the case of the *Fusarium* wilt disease of cotton, acclimatised American cottons are immune to wilt due to *Fusarium vasinfectum* Atkinson, in wilt sick soils in India. Indeed an investigation by the writer has shown that even American cotton varieties that

are highly susceptible to this disease under American conditions are immune under Indian conditions. The nature of the soil and the environment play therefore an important role in producing disease in plants by soil-inhabiting fungi.

Park and Bertus [1932] state that in Burma, *S. oryzae* is very common in paddy fields but does not cause any serious disease in the rice crop. Even with their successful pot experiments they state that under normal conditions of growth, parasitism of this fungus is not very marked, but the conditions favouring the disease do not seem to be very clear to them.

There is nothing to show, however, that when the host is introduced in a new country, the same parasite may not become a highly destructive factor against the successful cultivation of the crop. But in the country where the crop has grown for thousands of years, the parasite and host evidently lie side by side without the former causing disease in the latter.

Because of these considerations and the experimental evidence that has been adduced, the conclusion is irresistible that *Sclerotium oryzae*, while attacking living plants, does not produce any disease symptoms under normal conditions in the rice crop in India.

VIII. Summary

1. Important differences in the symptoms of rice diseases due to *Sclerotium oryzae* as described by different investigators are pointed out.

2. Cultures of spp. of *Sclerotium* assembled from various sources could be grouped into four categories, and this paper deals with the morphology and parasitism of two of them.

3. One *Sclerotium* possessed coloured hyphae, small smooth sclerotia and had the ability to change the colour of some substrates and was identified as *S. oryzae*. The cultures produced conidia of *Helminthosporium sigmoideum* but not the ascigerous stage. By analogy, however, it is considered that this fungus is *Leptosphaeria salvinii*.

4. The other (? *S. oryzae* non-chromogenic strain of Park and Bertus)* had hyaline mycelium, larger sclerotia with a rough surface, was unable to change the colour of substrates and was identified as *Rhizoctonia microsclerotia*.

5. *S. oryzae* failed to bring about any disease in pots or fields heavily infested with sclerotia. Sclerotia were however noted in the leaf sheaths and culms after the crop was harvested. In tests conducted in heavily infested soils negative results have been invariably obtained. In test-tube experiments, however, the fungus was able to produce the disease.

6. Pot tests with *R. microsclerotia* also gave negative results.

* See Appendix.

7. *S. oryzae* occurs in rice fields and the sclerotia can be seen infesting the sheaths and culms of healthy plants. Some plants with excessive tillers had sclerotial infestation while a large number did not have such infection.

8. Sterility could not be correlated with the presence of this fungus for both sterile and healthy plants showed infection and at the same time a large number of sterile plants did not show any infection.

9. The conclusion is therefore drawn that under normal Indian conditions these fungi are unable to produce disease in the rice crop.

IX. Appendix

Since the manuscript of this paper was finally prepared, Park and Bertus have published further results of their investigations on the sclerotial diseases of rice in Ceylon (*A. Roy. Bot. Gard. Peradineya*, 12 : 1-36, 1934). In part 3 of this communication they deal with a disease due to a strain of *Rhizoctonia solani* Kuhn which they designate ' A strain ', for it displayed certain constant differences from *R. solani*. This fungus has no bearing on any of the sclerotial fungi included in this paper. In part 4 they deal with *Sclerotium oryzae*, A strain and in part 5 with another *Rhizoctonia* which they call *R. solani*, B strain. A perusal of their paper and an examination of the plates revealed the fact that of the *Sclerotium* spp. sent by Park, the one considered in this paper as *S. oryzae*, A strain, non-chromogenic, was really their *R. solani*, B strain.

Their observations with regard to the growth in culture of this fungus and the sclerotial measurements are in accord, in major respects, with those obtained here. The only characteristic which this last named fungus has in common with *R. solani* is the mode of branching and the constriction at the junction of the branch with the parental hyphae. Typical *R. solani* discolours the medium when grown on potato-dextrose agar, giving the substrate a brownish tinge. Isolates No. 8, 9, 10 and 11 do not do so and the medium on the contrary retains its original tint. The sclerotia of *R. solani* are furthermore diffuse and crust like, while those of this fungus are compact, definite in form and oval to globose in shape. The cells of the medulla are also globose in the case of this rice *Rhizoctonia* and do not show the branched hyphal character of the medullary cells of *R. solani*. Park and Bertus do not seem disposed to consider this as a species different from *R. solani* as it differs only in a few respects and they prefer to call it B strain of it. But on the suggestion of Mr. Ashby this fungus has been identified here as *Rhizoctonia microsclerotia* Matz which identification has been confirmed by Matz himself. It is manifest therefore that *Rhizocotonia solani*, B strain of Park and Bertus is *R. microsclerotia* Matz.

As regards the pathogenicity of this fungus, Park and Bertus failed to induce it to parasitise rice seedlings growing in test tubes. In their pot experiments they state that only four out of a total of twenty-two plants died. It should be noted that the plants were not completely killed as new tillers were put forth by the plants, that only the central shoot had succumbed and that the tests were carried out in unsterilised soil, so that the parasitism of the fungus cannot be considered as settled.

are highly susceptible to this disease under American conditions are immune under Indian conditions. The nature of the soil and the environment play therefore an important role in producing disease in plants by soil-inhabiting fungi.

Park and Bertus [1932] state that in Burma, *S. oryzae* is very common in paddy fields but does not cause any serious disease in the rice crop. Even with their successful pot experiments they state that under normal conditions of growth, parasitism of this fungus is not very marked, but the conditions favouring the disease do not seem to be very clear to them.

There is nothing to show, however, that when the host is introduced in a new country, the same parasite may not become a highly destructive factor against the successful cultivation of the crop. But in the country where the crop has grown for thousands of years, the parasite and host evidently lie side by side without the former causing disease in the latter.

Because of these considerations and the experimental evidence that has been adduced, the conclusion is irresistible that *Sclerotium oryzae*, while attacking living plants, does not produce any disease symptoms under normal conditions in the rice crop in India.

VIII. Summary

1. Important differences in the symptoms of rice diseases due to *Sclerotium oryzae* as described by different investigators are pointed out.

2. Cultures of spp. of *Sclerotium* assembled from various sources could be grouped into four categories, and this paper deals with the morphology and parasitism of two of them.

3. One *Sclerotium* possessed coloured hyphae, small smooth sclerotia and had the ability to change the colour of some substrates and was identified as *S. oryzae*. The cultures produced conidia of *Helminthosporium sigmoideum* but not the ascigerous stage. By analogy, however, it is considered that this fungus is *Leptosphaeria salvinii*.

4. The other (? *S. oryzae* non-chromogenic strain of Park and Bertus)* had hyaline mycelium, larger sclerotia with a rough surface, was unable to change the colour of substrates and was identified as *Rhizoctonia microsclerotia*.

5. *S. oryzae* failed to bring about any disease in pots or fields heavily infested with sclerotia. Sclerotia were however noted in the leaf sheaths and culms after the crop was harvested. In tests conducted in heavily infested soils negative results have been invariably obtained. In test-tube experiments, however, the fungus was able to produce the disease.

6. Pot tests with *R. microsclerotia* also gave negative results.

* See Appendix.

7. *S. oryzae* occurs in rice fields and the sclerotia can be seen infesting the sheaths and culms of healthy plants. Some plants with excessive tillers had sclerotial infestation while a large number did not have such infection.

8. Sterility could not be correlated with the presence of this fungus for both sterile and healthy plants showed infection and at the same time a large number of sterile plants did not show any infection.

9. The conclusion is therefore drawn that under normal Indian conditions these fungi are unable to produce disease in the rice crop.

IX. Appendix

Since the manuscript of this paper was finally prepared, Park and Bertus have published further results of their investigations on the sclerotial diseases of rice in Ceylon (*A. Roy. Bot. Gard. Peradineya*, 12 : 1-36, 1934). In part 3 of this communication they deal with a disease due to a strain of *Rhizoctonia solani* Kuhn which they designate ' A strain ', for it displayed certain constant differences from *R. solani*. This fungus has no bearing on any of the sclerotial fungi included in this paper. In part 4 they deal with *Sclerotium oryzae*, A strain and in part 5 with another *Rhizoctonia* which they call *R. solani*, B strain. A perusal of their paper and an examination of the plates revealed the fact that of the *Sclerotium* spp. sent by Park, the one considered in this paper as *S. oryzae*, A strain, non-chromogenic, was really their *R. solani*, B strain.

Their observations with regard to the growth in culture of this fungus and the sclerotial measurements are in accord, in major respects, with those obtained here. The only characteristic which this last named fungus has in common with *R. solani* is the mode of branching and the constriction at the junction of the branch with the parental hyphae. Typical *R. solani* discolours the medium when grown on potato-dextrose agar, giving the substrate a brownish tinge. Isolates No. 8, 9, 10 and 11 do not do so and the medium on the contrary retains its original tint. The sclerotia of *R. solani* are furthermore diffuse and crust like, while those of this fungus are compact, definite in form and oval to globose in shape. The cells of the medulla are also globose in the case of this rice *Rhizoctonia* and do not show the branched hyphal character of the medullary cells of *R. solani*. Park and Bertus do not seem disposed to consider this as a species different from *R. solani* as it differs only in a few respects and they prefer to call it B strain of it. But on the suggestion of Mr. Ashby this fungus has been identified here as *Rhizoctonia microsclerotia* Matz which identification has been confirmed by Matz himself. It is manifest therefore that *Rhizocotonia solani*, B strain of Park and Bertus is *R. microsclerotia* Matz.

As regards the pathogenicity of this fungus, Park and Bertus failed to induce it to parasitise rice seedlings growing in test tubes. In their pot experiments they state that only four out of a total of twenty-two plants died. It should be noted that the plants were not completely killed as new tillers were put forth by the plants, that only the central shoot had succumbed and that the tests were carried out in unsterilised soil, so that the parasitism of the fungus cannot be considered as settled.

They admit that the fungus is weakly parasitic and that it is not clear to them whether under field conditions it was responsible for the two outbreaks that had been reported, infection being absent in subsequent crops raised in the same fields.

The description and the sclerotial measurements of the fungus, which they have described as *S. oryzae*, A strain, tally with those of an isolate which was received by the writer from Nakata under the name *Sclerotia microsphaeroides* Nakata. Park and Bertus' *S. oryzae*, A strain does not have any of the characteristics common to all the different strains of *Sclerotium oryzae*. It has sclerotia which are not easily separable, with rather rough surface and with a much smaller size, and in these respects it agrees perfectly with Nakata's *Sclerotium*. Furthermore as *S. oryzae* has now been shown to be a stage of *H. sigmoideum* and *L. salvinii*, a sclerotium differing from this to the extent *S. oryzae*, A strain does, cannot be considered as a strain of that fungus. Perhaps the name given by Nakata, *Sclerotium microsphaeroides* would be more appropriate.

X. Acknowledgments

The writer's thanks are due to Mr. K. Amrithrao, Librarian, Indian Institute of Science, Bangalore, for kindly translating into English the articles by Cattaneo, and Briosi and Cavara. Appreciation is expressed to the plant pathologists abroad for sending the cultures of sclerotial fungi, especially to Dr. E. C. Tullis who arranged to send seeds of susceptible varieties of rice from U. S. A. Finally grateful acknowledgment is made for the valuable help rendered by Mr. S. F. Ashby of the Imperial Mycological Institute, Kew, England, in identifying the cultures.

REFERENCES

Bertus, L. S. (1929). *Rept. Myc. Div. Ceylon. Dept. Agric. Tech. Rept.* for 1928, 5.

Briosi, G. and Cavara, F. (1886). I Funghi parassiti Piante cultivate od utili. Fasc. I. Pavia, pl. 25.

Butler, E. J. (1913). *Agric. Res. Inst. Pusa, Bull.* 134.

Cattaneo A. (1879). *Arch. Triene Lab. Critt. Pavia* 2-3, 75-83.

Duggar, B. M. and Davis, A. M. (1919). *Ann. Mo. Bot. Gard.* **6**, 159-170.

Fisher, R. A. (1932). 'Statistical Methods for Research Workers', London.

Jones, L. W. (1930). *J. Amer. Soc. Agron.* **22**, 861-867.

Massa, C. (1912). *Ann. di Botanica* **10**, 417-430.

Matz, J. (1917). *Phytopath.* **7**, 110-118.

——— (1921). *J. Dept. Agric. Porto Rico* **5**, 5-34.

Mundkur, B. B. (1926). *J. Ind. Bot. Soc.* **5**, 9-10.

——————— (1934). *Ind. J. Agric. Sci.* **4**, 758-778.

Park, M. and Bertus, L. S. (1932). *Ann. Roy. Bot. Gard. Perad.* **11**, 343-359.

Reyes, C. M. (1929). *Philip. Agric. Rev.* **22**, 313-331.

Shaw, F. J. F. (1913). *Mem. Dept. Agric. Ind., Bot. Ser.* **6**, 11-23.

Tisdale, W. H. (1921). *J. Agric. Res.* **21**, 649-658.

Tullis, E. C. (1933). *J. Agric. Res.* **47**, 675-687.

Tullis, E. C. and Cralley, E. N. (1933). *Ark. Agr. Expt. Stat. Bul.* **295**.

Vincens, F. (1923). *Rev. Path. Veg. et Ent. Agr.* **10**, 112-131. (Original not seen *Rev. App. Myc.* **3**, 174. 1924).

SOME OBSERVATIONS ON THE ESSENTIAL OIL CONTENT OF *ROSHA* GRASS (*CYMBOPOGON MARTINI* VAR. *MOTIA*)

BY

GIRDHARI LALL, PH.D. (LOND.), D.I.C.,

Research Assistant, Chemical Research Laboratories, Punjab Agricultural College, Lyallpur

(Received for publication on 7th December 1934)

(With three text-figures)

Rosha grass on steam distillation yields an essential oil known as Palmarosa oil or Rusa oil which is used as a scent for soaps and also in the manufacture of various perfumes. About 230 acres of land near Jaranwala (Punjab) are under this crop. The grass was planted about eight years ago ; and the crop at about six inches above ground level is cut annually for distillation in the winter (October to December). The part remaining above ground is burnt and a fresh crop sprouts in the month of March.

In past years it was observed that if the crop was not distilled before the onset of frost, the oil content was seriously affected and it seemed likely that cutting the grass and stacking it before the appearance of frost might arrest the decline in the oil content. The investigation here described was therefore started with the object of determining the amount of oil in different parts of the plant, leaves, flowers, and stalks ; the effect of frost on the seasonal variation of the oil content in different parts, and the effect on the oil content of harvesting and stacking the crop before frost.

The investigation lasted from August 1932 to March 1933.

Material and methods of analysis

I. MATERIAL AND SAMPLING

Half an acre of grass planted four years previously was reserved and a composite sample obtained from twenty to thirty different places—carefully packed in a bag to avoid damage to leaves and flower-heads and also to minimise the loss of moisture—was brought to the laboratory for analysis. About eighteen hours elapsed between the sampling at the farm and the time of analysis. Leaves, stalks and flower-heads were carefully separated, cut into small pieces, and kept separately after thorough mixing. Another portion consisting of the entire plant was similarly treated.

II. Methods of analysis

(*a*) *Dry weight*

Individual samples as obtained above were dried to constant weight at 100°C. for 24 hours.

(*b*) *Essential oil*

Samples of leaves, stalks, flowers and the entire plant from 100 to 800 grams in weight were distilled in steam for two hours, and the distillate received directly into a large separating funnel. The distillation was complete after about one hour and a half, when no more oil came over. The water which settled at, and was constantly removed from, the bottom of the separating funnel during the distillation wa turbid, and on shaking with ether gave only a negligible amount of oil, while the oily layer from the separating funnel was transferred to a small tared separating funnel. In order to remove traces of moisture a small test tube (made of thin tin foil) containing anhydrous sodium sulphate was kept suspended overnight in the funnel. This treatment dried the oil to a constant weight. The dried oil was then weighed. The percentage of oil given in the following tables has been calculated on the dry weight of the material analysed.

(*c*) *Chemical and physical examination of the oil*

The analysis consisted of the following determinations :—

1. Specific gravity.
2. Refractive index.
3. Optical rotation.
4. Solubility.
5. Acid value.
6. Ester value.
7. Ester value after acetylation.
8. Free alcohol (as geraniol)
9. Total alcohol (as geraniol)

All the determinations were carried out after the manner of Gildemeister and Hoffmann [1913], which may be described briefly as below :—

1. Specific gravity.—Specific gravity was determined at room temperature with a pyknometer and the figures obtained reduced to 15°C. by applying a correction coefficient of 0·00075 per each degree of difference added or subtracted as the temperature was above or below 15° C. [Villavecchia, 1918].

2. Refractive index was determined by Abbe's refractometer at room temperature and corrected to 15°C. by 0·00035 factor for each degree of temperature [Gildemeister and Hoffman, 1913].

3. Optical rotation was determined by polarimeter in a one decimeter tube at room temperature.

4. Solubility.—Five to ten c.c. of the oil were placed in 100-c.c. stoppered graduated cylinders and alcohol (70 per cent by volume) was added in small portions, with vigorous shaking, till all the oil was dissolved.

5. Acid value, 6. Ester value.—To about two grams of accurately weighed oil in a 500-c.c. flask, about twice its volume of neutral alcohol was added. The mixture was carefully titrated with semi-normal potassium hydroxide solution using phenolphthalein as indicator. After the neutralisation of the free acid, 10 c.c. of half-normal solution of alcoholic caustic potash were added and the mixture heated on a water bath under a reflux condenser for one hour, and on cooling the excess of alkali titrated with semi-normal sulphuric acid. The acid and ester values were calculated from the first and the last titrations respectively.

7. Ester value after acetylation.—Ten c.c. of oil mixed with an equal volume of acetic anhydride and about two grams of dry sodium acetate were boiled under a reflux condenser for one hour, cooled, a small quantity of water added to the flask, and the mixture again heated for 15 minutes on the water bath to decompose the excess of acetic anhydride. The oil was separated in a separating funnel, washed with water until neutral and dried with anhydrous sodium sulphate. About two grams of the acetylated oil were saponified as above and the free acid having been previously neutralised, the ester value of the acetylized oil was determined.

8. Free alcohol, 9. Total alcohol (geraniol).—These were calculated from the ester values before and after acetylation of the oil from tables given in "The Volatile Oils" by Gildemeister and Hoffmann. While calculating these values, the ester in the original oil has been included.

Experimental results

A. Changes in the dry weight of different parts of the plant during growth and maturity

The dry weights of the entire plant and its different parts are given in Table I and graphically shown in Fig. 1. It will be noticed that the dry weight in all cases continues to increase upto the end of September when it shows a decline lasting till about the end of October when, synchronising with the appearance of flowers it suddenly rises to be followed by a gradual fall till the beginning of December. When, however, a severe attack of frost is associated with a sudden rise in the dry weight, which is more pronounced in the flower-heads, this rise in all the cases continues till the end of the season. Attack by frost rendered the entire crop brown, with consequent loss of moisture resulting in a higher percentage of total solids. This effect was more pronounced in the case of the flower-heads, probably owing to their being exposed directly to frost.

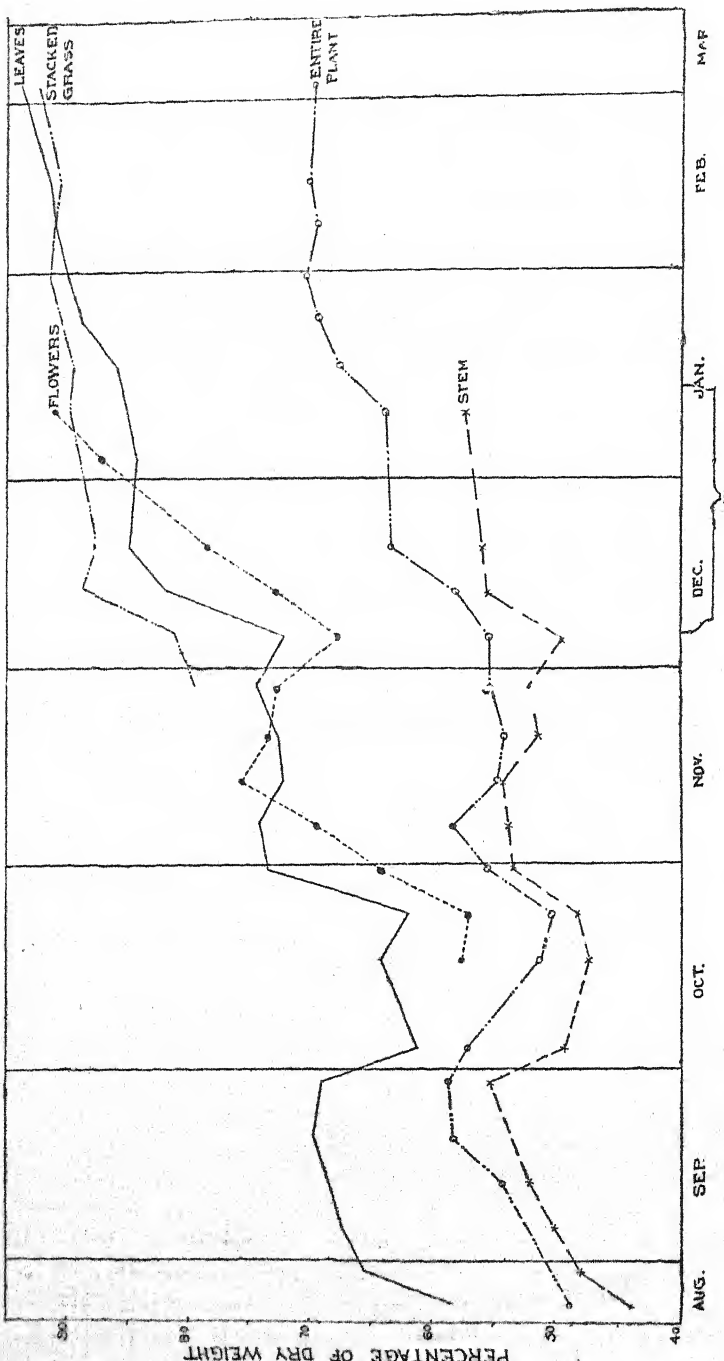


Fig. 1. Variations in dry weight of Rosha grass (*Cymbopogon Martini* var. *Motia*) throughout the season.

(B) Seasonal variations in the essential oil content of leaves, stalks, flower-heads and the entire plant

1. Essential oil in leaves

The essential oil data are given in Table I and graphically represented in Fig. 2. It is seen that the oil-content of leaves ranges between 1·22 and 1·30 per cent of the dry weight in the month of September, and gradually rises to a maximum of 1·39 per cent by the middle of October, when flowers appear, after which it shows a slow gradual decline till the end of November. After frost the oil content shows a further decrease, the net loss being 13·0 per cent of the maximum oil content. With a further advance in the season (by the beginning of March) the oil content gradually falls to a minimum of 0·77 per cent about the beginning of March, thus showing a decrease of about 45 per cent from the maximum.

2. Essential oil in flower-heads

Flowers appear in the second week of October and by the bginning of 3rd week 50 per cent of the plants were in flowers. The oil content of the flowers reaches a maximum of 1·37 per cent (on dry weight) by the end of October and then declines. The oil content shows a sudden decline after frost, a figure of 0·59 per cent by the second week of January when it falls still further, showing at this time a total decrease of 59 per cent from the maximum.

3. Oil-content in stalks

From Table I it is seen that the oil content in stalks is negligible, being only 0·03 per cent (on dry weight) during the month of September. Determinations of the oil content of stalks were discontinued from October onwards, as it was noticed that, with the advance of the season the stalks were thickening and becoming tough.

TABLE

Dry weight and essential oil contents of Rosha grass

Date	Dry weight per 100 grms. of fresh weight (Material dried at 100°C. for 24 hours)					Grms. of essential oil per 100 grms. fresh weight of the material analysed		
	Leaves	Stalks	Heads (Flower)	Entire plant	Stacked grass	Leaves	Stalks	Flower-heads
24th August 1932 .	58·4	44·0	...	48·8	...	0·722	0·011	...
29th August 1932 .	65·4	48·0	...	...	...	0·796	0·016	...
5th September 1932.	67·4	50·0	...	...	...	0·873	0·013	...
12th September 1932	63·6	52·0	...	54·2	...	0·821	0·018	...
19th September 1932	69·2	50·6	...	58·4	...	0·818	0·013	...
28th September 1932	69·0	55·2	...	58·2	...	0·829	0·014	...
3rd October 1932 .	61·2	49·2	...	57·4	...	0·807	0·014	...
17th October 1932 .	64·2	47·4	57·6	51·2	...	0·893	...	0·768
24th October 1932 .	61·8	48·2	57·0	50·2	...	0·853	...	0·780
31st October 1932 .	73·2	53·4	64·0	55·4	...	0·981	...	0·876
7th November 1932	74·0	53·6	69·2	58·4	...	0·915	...	0·864
14th November 1932	72·0	54·4	75·5	54·6	...	0·964	...	0·926
21st November 1932	72·4	51·2	73·0	54·2	...	0·948	...	0·874
28th November 1932	74·2	52·0	72·6	55·2	79·2	0·916	...	0·868
6th December 1932	69·8	55·4	67·4	55·2	80·6	0·864	...	0·788
13th December 1932	81·6	55·8	72·5	58·0	88·6	0·985	...	0·773
20th December 1932	84·6	51·2	78·2	63·3	87·4	1·037	...	0·879
3rd January 1933 .	84·0	57·4	86·8	59·2	84·6	1·087	...	0·956
10th January 1933 .	89·2	56·0	90·8	63·6	89·6	0·917	...	0·528
17th January 1933 .	85·6	...	89·2	67·4	89·2	0·899	...	0·510
25th January 1933 .	88·7	...	...	69·3	90·5	0·874	...	...
31st January 1933 .	89·8	...	...	70·2	91·4	0·815	...	...
8th February 1933	90·8	...	...	69·2	90·6	0·762	...	...
15th February 1933	91·2	...	...	69·8	90·2	0·715	...	...
2nd March 1933 .	93·6	...	...	69·4	92·2	0·718	...	...

I.

(Cymbopogon Martini *var.* Motia) *Season 1932-33*

Essential oil content calculated to 100 grms. dry weight of the material analysed							Remarks
Entire plant	Stacked grass	Leaves	Stalks	Flower-heads	Entire plant	Stacked grass	
0·155	...	1·236	0·025	...	...	...	
...	...	1·218	0·033	...	...	...	With the advance of season the leaves above the root were drying and becoming brown.
...	...	1·295	0·026	...	...	...	
0·148	...	1·291	0·035	...	0·273	...	
0·161	...	1·183	0·026	...	0·276	...	
0·164	...	1·302	0·025	...	0·282	...	
0·164	...	1·319	0·028	...	0·286	...	
0·198	...	1·391	...	1·334	0·386	...	About 50 per cent of the plants in the plot were flowering.
0·199	...	1·380	...	1·368	0·395	...	Maturity period.
0·222	...	1·340	...	1·369	0·400	...	
0·210	...	1·332	...	1·249	0·307	...	
0·202	...	1·338	...	1·228	0·370	...	
0·191	...	1·311	...	1·200	0·365	...	The crop was cut on 21st November and dried in field and stacked on 26th Nov. 1932.
0·192	0·237	1·289	...	1·246	0·348	0·300	
0·187	0·248	1·238	...	1·171	0·339	0·308	Severe attack of frost.
0·162	0·223	1·207	...	1·067	0·279	0·252	
0·189	0·220	1·226	...	1·125	0·299	0·252	Intermittent attacks of mild and severe frost.
0·179	0·215	1·294	...	1·101	0·302	0·254	
0·186	0·225	1·028	...	0·592	0·292	0·251	Intermittent attacks of frost.
0·183	0·219	1·050	...	0·562	0·272	0·246	
0·180	0·230	0·985	...	...	0·260	0·254	
0·171	0·242	0·907	...	...	0·244	0·265	
0·165	0·230	0·839	...	...	0·238	0·254	
0·157	0·248	0·785	...	...	0·225	0·275	
0·127	0·224	0·767	...	...	0·183	0·243	

4. Oil-content of the entire plant

Seasonal changes in the oil content of the entire plant are similar to those of the leaves and flowers. During the time preceding the maturity period (third week of October to end of November) the oil content in the plant ranges from 0·15 to 0·22 per cent on the fresh weight and 0·27 to 0·40 on the dry matter. The period of maximum oil content in the entire plant synchronises with that in the leaves and flowers. With frost during the first week of December a sudden fall — though not as great as in the leaves—in the oil content is observed. Later on with intermittent attacks of frost and advance of season the oil content shows a gradual decline. By the end of the second week of January, when frost ceased, a decrease of 32 per cent from the maximum was observed, whereas by the beginning of March the decrease was about 54 per cent from the maximum.

C. THE EFFECT ON THE OIL-CONTENT OF STACKING THE GRASS BEFORE FROST

The fully matured crop from half the plot reserved for the investigation was harvested before the onset of frost on the 21st November, and in order to avoid fermentation during stacking, it was previously dried in the sun for four days. For the estimation of oil about thirty representative samples from the field were collected, carefully packed in muslin bags to avoid injury to the flower-heads and leaves, and placed in the above-mentioned stack of grass on the 26th of November. The oil content of these samples and of the corresponding standing crop in the field was periodically examined. The data of dry weight and essential oil content of the stacked grass are given in Table I and graphically shown in Figs. 1 and 2. As seen from Fig. 1 the dry weight shows a rapid increase till the middle of December, after which it decreases till the maximum figure is obtained by the beginning of March.

It will be seen from Fig. 2 that the loss in oil content in the stacked grass takes place only in the first two weeks of stacking after which the oil content remains practically constant over a period of three months when the analyses were discontinued.

TABLE II

Showing the comparison of the essential oil content of the standing Rosha grass in the field and the corresponding stacked grass

Date	Standing crop in the field				Stacked grass		
	Dry weight per cent I	Per cent essential oil on fresh weight II	Per cent of essential oil calculated on initial fresh weight III	Dry weight per cent IV	Per cent essential oil on fresh weight V	Per cent essential oil calculated on initial fresh weight VI	Loss of oil in the stacked grass (0·191—figures of the previous column)
21st November 1932	54·2	0·191	...	...	...	...	...
28th November 1932	55·2	0·192	0·188	79·2	0·237	0·162	·029
6th December 1932 .	55·2	0·187	0·184	80·6	0·248	0 167	024
13th December 1932 .	58·0	0·162	0·151	88·6	0·223	0·136	·055
20th December 1932.	63·3	0·189	0·162	87·4	0·220	0·136	·055
3rd January 1933 .	59·2	0·179	0·164	84·6	0·215	0·138	·053
10th January 1933 .	63·6	0·186	0·158	89·6	0 225	0·136	·055
17th January 1933 .	67·4	0·183	0·147	89 2	0·219	0·133	·058
25th January 1933 .	69·3	0·180	0·141	90·5	0·230	0·138	·053
31st January 1933 .	70·2	0·171	0·132	91·4	0·242	0·143	·048
8th February 1933 .	69·2	0·165	0·129	90·6	0·230	0·138	·053
15th February 1933 .	69·8	0·157	0·122	90·2	0·248	0·149	·042
2nd March 1933 .	69·4	0·127	0·099	92·2	0·224	0·132	·059

Table II gives the oil content of the standing crop in the field, together with the corresponding figures for the stacked grass. Periodic determinations of the oil content of fresh and the stacked grass have been calculated on the intial fresh weight of the green crop as it was noted on 21st October, the date of cutting the crop for stacking. Thus figures of columns III and VI are the actual amounts of oil per 100 grams of intial fresh weight of the green and stacked grass respectively. It is clear from the figures of these two columns that the standing crop in the field, though subjected to intermittent attacks of frost from the second week of December to the middle of January, contains essential oil in excess of that in the stacked grass uptil the 25th of January 1933 and thereafter the stacked grass maintains a higher oil content than the crop standing in the field. In order to see whether the differences in the essential oil content of the standing and the stacked grass are significant, Fisher's [1930] 't'-test of significance has been applied to the figures of columns III and VI (upto the 25th of January 1933). The observed value of 't' is 6·65 (while the one per cent value of 't' for $n = 7$ is only 3·499) showing that the differences in the oil content of the standing green grass and the corresponding stacked grass are clearly significant. It is evident, therefore, that

the standing crop, though affected by frost yielded more oil than the stacked grass till the end of January, whereas due to the advance of the season and adverse climatic conditions, the standing crop dried up with a considerable decrease in the oil content, which in the month of February was only 84 per cent of the oil present in the stacked grass at this time.

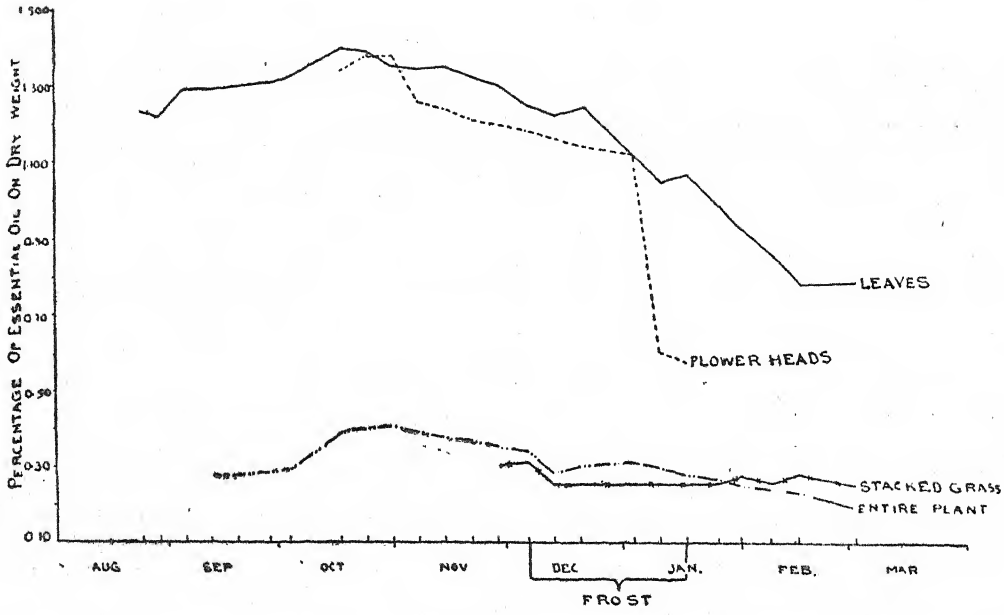


Fig. 2. Seasonal variation in the essential oil content of Rosha grass (*Cymbopogon Martini* var. *Motia*) dry weight basis

The loss in oil-content of the grass during the period of stacking is shown in Fig. 3 and Table II. The values in column VII have been plotted against time. From the table it is seen that the stacked grass shows a decrease in the oil content from 0·191 (oil present in the grass on the day on which it was harvested for stacking) to an average figure of 0 164 uptil the 28th of November, showing a decrease of 14 per cent and a further decrease of about 17 per cent in the oil content till the 6th of December after which it remained almost constant till the beginning of March. These points are further illustrated by a study of Fig. 3 from which it is seen that a rapid loss in the oil content of the stacked grass occurred till the end of the first week of December and thereafter it maintained almost a constant level with only slight fluctuations (due probably to experimental error) till the end of the period of stacking.

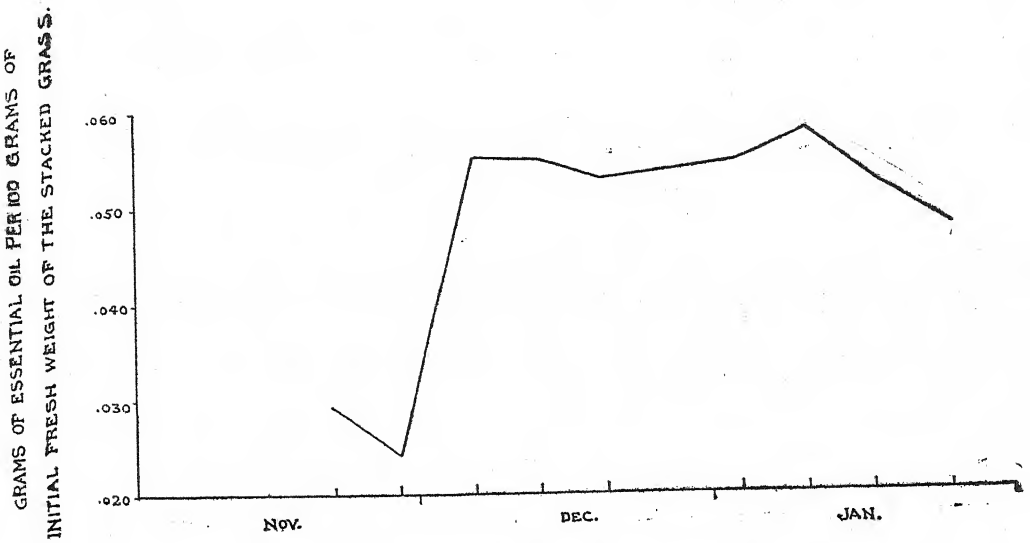


Fig. 3. Loss of essential oil in the stacked grass

D. Chemical composition of palmarosa oil

Table III gives analyses of oil obtained from different sources. No appreciable variation in the constants of the above samples is observed except in the acid value, the ester value and the geraniol content. It will be seen that with the exception of sample 2 all the remaining samples gave a high acid value; sample 3 giving the highest figure. The high acid and ester values in sample 3 indicate a greater stability of esters and free fatty acids during the process of steam distillation as carried out in the laboratory, thus resulting in a slightly lower content of free geraniol and a comparatively high content of total geraniol. It is clear from the free and total geraniol figures (93·5 to 96·06 per cent respectively) that the leaves yielded an oil of the best quality, while the stacked grass gave the poorest quality oil. The oil obtained both from stalks and flower-heads (which contains mostly the woody stalk) is almost similar in quality.

TABLE III

Physical and chemical constants of palmarosa oil obtained from different sources

Sample	Specific gravity at 15°C.	Refractive index at 15°C.	Optical rotation	Solubility Vol. of alcohol (70 per cent. vol.) dissolving one volume of oil	Acid value	Ester value	Ester value after acetylation	Free alcohol (geraniol) per cent	Total alcohol (geraniol) in the original oil per cent
1. 1931. Farm distilled	0·8884	1·4789	+0·025	1·45	1·13	13·43	272·67	88·50	92·19
2. 1932. Farm distilled .	0·8887	1·4789	+0·030	1·52	0·65	12·39	274·85	89·86	93·22
3. Laboratory distilled (1932).	0·8931	1·4790	...	1·60	1·57	27·93	283·83	87·09	94·78
4. From leaves Farm distilled, 1932.	0·8891	1·4781	+0·005	1·41	1·11	9·33	280·28	93·52	96·06
5. Oil from flower-heads (1932) Farm distilled.	0·9001	1·4780	+0·030	1·36	1·24	10·45	276·86	91·56	94·43
6. Oil from stalks (1932) Farm distilled.	0·8895	1·4781	+0·005	1·39	1·04	14·28	279·62	91·10	95·03
7. Oil from stacked grass (1932) Farm distilled.	0·8877	1·4791	+0·010	1·50	1·07	15·09	272·73	87·82	91·98

N.B.—In calculating the amount of total and free geraniol the esters in the original oil have been taken into consideration.

Discussion

It is evident from the data presented in the preceding pages that the leaves yield the highest and the largest amount of oil throughout the season.

The flowers (appearing in the third week of October) show a maximum oil content during the fourth week of October, a period which is associated with the maximum of oil content both in the leaves and in the entire plant. Thus the grass is fully mature during the fourth week of October, whereas the oil content in all cases (leaves, flower-heads and the plants) showed a slow gradual decrease during the month of November. The onset of frost, which followed, affected the oil content to a marked degree. These observations regarding the essential oil content of the plant during growth and maturity (before the attack of frost) are in accordance with the work of Reilly and Taylor [1927] and Hogstad [1926] who worked on the "Oil of Peppermint"; and "The Origin, Nature, and Physiological Roll of the Essential Oil of American Wormwood," respectively. These workers have also found that the highest yields of oil are obtained during the period of flowering, and also that the largest amount of oil is obtained from the leaves and flowering tops which show a gradual decrease in the oil content as the plant reaches maturity.

The most suitable time for distilling the oil seems to extend from the fourth week of October to the third week of November, a period which corresponds with the maximum oil content of the plant.

In regard to the effect of stacking on the oil content, as has been already shown, the percentage decrease up to the first week of January is less in the case of the standing crop, and removal and stacking of the crop before frost does not arrest the decline in the oil content. It is, therefore, suggested that to obtain a maximum yield of oil the crop should be harvested and the oil distilled before the frost.

V. Summary

Seasonal variations in the oil content of *Rosha* grass (*Cymbopogon Martini* var. *motia*) and its different parts, *viz.*, leaves, flowers and stalks have been studied from August 1932 to March 1933.

The leaves yielded the largest amount of oil throughout the season. Flowers are short-lived and gave the maximum amount of oil (1·37 per cent on dry weight) during the fourth week of November (a week after their appearance), the stalks contain only a negligible amount of oil (0·03 per cent on dry weight).

The whole plant yielded the maximum amount of oil about a week after flowering. The time extending from the fourth week of October and the third week of November has been found to be the ' maturity ' period and best suited for harvesting the crop and distillation of the oil.

Intermittent attacks of frost render the plant brown and decrease the oil content of the leaves, flowers and the entire plant to the extent of 13 per cent, 59 per cent and 32 per cent, respectively. The flower-heads are more susceptible to frost.

Stacking the grass before frost does not arrest the decline in the oil content. The crop, for distillation purposes, should therefore be harvested before the onset of frost.

Chemical examination of the oil indicates that the leaves yield the best quality oil. The flower-heads (containing the woody stalk), and the stalks give an oil of a slightly inferior quality, whereas the stacked grass yields the poorest quality oil.

The writer wishes to thank Dr. P. E. Lander and Dr. Ramji Narain for their valuable criticism and advice, during the course of the investigation.

He is also thankful to Rai Sahib Jai Chand Luthra for his helpful suggestions and his kindness for providing necessary facilities for work at the Rosha Grass Farm.

REFERENCES

Fisher, R. A. (1930). " Statistical Methods for Research Workers."

Gildemeister and Hoffmann (1913). " The Volatile Oils," 2nd Edition, Vol. I.

Hogstad, Jun. A. (1926). *Amer. J. Pharm.*, 98.

Reilly and Taylor (1927). " *Econ. Proc. Roy. Dublin Society*, 2.

Villavecchia (1918). " Applied Analytical Chemistry, Vol. II."

STUDIES ON *RHIZOBIUM LEGUMINOSARUM* OF BERSEEM (*TRIFOLIUM ALEXANDRINUM*)

BY

M. R. MADHOK, B. Sc.,

Bacteriological Assistant, Agricultural Research Institute, Lyallpur

(Received for publication on the 14th February 1935)

INTRODUCTORY

Because of its high yields and great nutritive value berseem or Egyptian clover is becoming increasingly popular in the Punjab as a valuable fodder.

It was found, however, that it will not do well at all places and investigations showed that this was due to the lack of nodule formation on the roots. The defect was remedied by artificial inoculation of such soils with the requisite bacteria [Sarkaria, 1933] and the treatment was instrumental in increasing the yields about 86 per cent on the average*.

In view of the importance which this fodder crop has assumed in the Punjab, a study of its root-nodule bacteria and an investigation into their cross inoculation relationship with the nodule bacteria already present in our soils is of deserving importance.

Nobbe and his co-workers [1895] came to the conclusion that the bacteria in the nodules of the various legumes were all strains of the same organism. It was soon recognised however that different leguminous plants require specific organisms for inoculation.

Burrill and Hansen [1917] by a series of cross inoculations demonstrated the existence of eleven kinds of nodule bacteria and divided these into three distinct groups.

Later, Löhnis and Hansen [1921] studied the nodule bacteria from fourteen different legumes and divided their fifty-seven cultures into two groups which differed morphologically as well as physiologically.

Fred and Davenport [1918] established differences in the critical pH for the growth of the nodule bacteria of the common legumes and arranged them into five groups.

---

* The average has been taken from the results of the inoculation experiments conducted at ten different places in the Punjab.

As a result of cross-inoculation studies Hansen [1921] divided the nodule bacteria of the various legumes into eleven distinct groups. The different members in any one group are those which can be inoculated by the strain of the nodule organism specific for that group. According to him all the *Trifolium* species are included in one group. The organism infecting the members of this group has been described as peritrichic.

On the basis of serological investigation, Klimmer and Kruger [1914] formed nine groups of legume bacteria. Later Bialosuknia and Klott [1923] noted that the same kind of plant from different sources may have nodule bacteria with different agglutinating properties. They also report that different nodules on the same plant may contain serologically different bacteria. This observation was later confirmed by Wright [1925] in the case of the soybean organism.

As will be clear from the above we have not yet been able to establish any morphological differences amongst the various groups of the nodule bacteria, except the division into two groups suggested by Löhnis and Hansen [1921]. We do not know whether we are dealing here with different species or mere biological races [Waksman, 1926].

For specific separation one has to depend on cross-inoculation and serological tests.

The agglutination test, which has been widely used in other fields of bacteriology for diagnostic purposes, has clearly shown that the nodule bacteria from even one legume could be separated into several groups. Hence in the present investigation only cross-inoculation and cultural methods have been used.

In addition to the cultural study of the four strains of the organism on some of the more important media, the effect of certain physical factors has also been investigated.

Experimental

Description of the organism

The organism is a gram-negative coccus or a rod, according to the stage in the cycle of its growth, varying from 1 to 3 μ in length and being about 1 μ in breadth. The organism when motile has one polar flagellum.

The organism studied was obtained from known cultures of bacteria isolated from the root nodules of berseem. These cultures had been tested for purity and ability to form nodules. All the cultures were further tested for the absence of *B. Radiobacter* on milk and potatoes according to the method of Löhnis and Hansen [1921].

The strains studied were freshly isolated from plants grown in different fields. For the cultural study four strains were used, while the other part of the investigation was mainly carried on with strain I.

Cultural characteristics

The cultural characteristics of the four strains were studied on a variety of different media. The rate and amount of growth were recorded from time to time. Besides nitrogen-free media a number of media containing nitrogen in addition to the various carbohydrates were also used since, contrary to the views previously held, it has now been shown by certain workers [Albrecht, 1920] that nodules are produced, and a satisfactory growth of the legumes does result, even when large amounts of organic nitrogen or nitrates are present in the soil.

The carbohydrate media used were prepared on three bases. Some were prepared on Ashby's solution as a base, others on soil extract and still others with beef extract broth. This was done with the object of providing no nitrogen (as in Ashby's) providing slight amounts of nitrogen (as in soil extract) and providing a good amount of nitrogen (as in beef extract).

The only differences noticed were in the rate and amount of growth as shown in Table I.

TABLE I

Showing cultural characteristics of the four strains. Media inoculated on 13th December 1931 and examined on 23rd December 1931.

Medium	Strain I			Strain II		
	Beef extract	Soil extract	Ashby's *	Beef extract	Soil extract	Ashby's
Glucose agar	+ + +	+ + +	+ +	+ +	+ +	+ +
Glucose broth	Slight haziness	Hazy growth	Slight haziness	Slight haziness	Hazy growth	Slight haziness
Lactose agar	+ + +	+ +	+	+ + +	+	+
Lactose broth	Slight haziness	Hazy growth	Slight haziness	Slight haziness	Hazy growth	Slight haziness
Saccharose agar	++++	+	+ +	+ + + +	+	+ +
Saccharose broth	Very slight reddening	Haziness, colour lightened	Slight haziness	Indicator colour lightened	Haziness, colour lightened	Slight haziness
Dextrin agar	+ + +	+	+	+ + +	+	+
Dextrin broth	Slight haziness	Colour deepened pink	Hazy growth	Slight deepening of the colour	Colour lightened	Hazy growth
Litmus milk	Serum zone on the top			Serum zone on the top. litmus deepened blue		Colour of

* Calcium carbonate had been added to the Bouillon cultures and so the change in indicator could not be studied.

\+ Slight growth
\+ + Good growth
\+ + + Heavy growth
\+ + + + Extra heavy growth
— No growth

TABLE I—(*contd.*)

Medium	Strain III			Strain IV		
	Beef extract	Soil extract	Ashby's *	Beef extract	Soil extract	Ashby's
Glucose agar	+ + +	+ +	+	+ + +	+ +	+ +
Glucose broth	—	Haziness, good growth	Slight haziness	Slight haziness	Good growth	Haziness
Lactose agar	+ + +	+	+	+ + + +	+	+
Lactose broth	Slight haziness	Hazy growth	Slight haziness	Slight haziness	Good growth	Very slight haziness
Saccharose agar	+ + + +	+	+ +	+ + + +	+	+ +
Saccharose broth	Slight haziness	Hazy, colour lightened	Slight haziness	Slight haziness	Growth, colour lightened	Slight haziness
Dextrin agar	+ + +	+	+	+ +	+	+ +
Dextrin broth	Slight haziness	Colour lightened	Haziness	Slight deepening of the colour	Growth	Slight growth
Litmus milk	Serum zone on the top. Colour of litmus deepened blue			Serum zone on the top. Colour of litmus deepened blue		

* Calcium carbonate had been added to the Bouillon cultures and so the change in indicator could not be studied.

Since the growth in broth cultures was not definite in all cases, transfers from these cultures on to soil-extract-mannite-agar slants were made with the following results :

Inoculated from		Growth			
		Strain I	II	III	IV
Glucose broth	Beef extract	+ve	—ve	Scanty	Scanty
	Soil extract	+ve	+ve	+ve	+ve
	Ashby's	+ve	+ve	+ve	+ve
Lactose broth	Beef extract	+ve	Scanty	Scanty	Scanty
	Soil extract	+ve	+ve	+ve	+ve
	Ashby's	+ve	+ve	+ve	+ve
Saccharose broth	Beef extract	+ve	+ve	Scanty	Scanty
	Soil extract	+ve	+ve	+ve	+ve
	Ashby's	+ve	+ve	+ve	+ve
Dextrin broth	Beef extract	+ve	+ve	Scanty	Scanty
	Soil extract	+ve	+ve	+ve	+ve
	Ashby's	+ve	+ve	+ve	+ve

+ve signifies ' growth '.
—ve signifies ' no growth '.

Scanty growth on the slants indicates the unsuitable nature of the broth medium in which the organism had existed under unfavourable conditions.

From the above the following conclusions can be drawn :

1. That for all the four strains tested the growth in agar cultures was the best on carbohydrate media with beef extract base.
2. That the growth on saccharose beef extract agar medium has been the best for all the four strains ; being followed in order, by lactose, glucose and dextrin, the last giving the poorest results.
3. Only a slight growth was noticed in all the broth cultures and of these the cultures on soil extract base proved to be the best.
4. A serum zone was visible in litmus milk for all the four strains, an increase in the blue colour of the litmus indicator showing the alkaline nature of the by-products formed.
5. There was no growth on potato for any of the strains.

Reaction tolerance of the organism

In order to test the reaction sensitiveness of the organism, soil extract mannite agar [Joshi, 1920] with pH varying from 3 to 10 was prepared, slanted and inoculated in duplicate from a week old culture.

On examination after a week's incubation, good growth of the organism was observed in media slants with pH 5·0, 5·4, 5·8, 6·0, 7·2, 8·0, 9·0 and 9·8. Growth, however, was absent in tubes with pH 3·0 and 4·0. This showed that the organism could tolerate an acidity corresponding to pH 5·0 but not further.

THERMAL DEATH POINT

(*i*) The thermal death point of the organism was determined by inoculating mannite solution tubes of an approximately equal bore. Each of the tubes contained 10 c.c. of the solution. The inoculated tubes were exposed in duplicate to different temperatures for ten minutes. The temperature range studied was from 46°C. to 70°C., with a two-degree step. The results are given below :—

Strain No.	Lowest temperature at which no growth occurred
I	54°C.
II	52°C.
III	52°C.
IV	52°C.

(*ii*) Since the organism has its natural habitat in the soil it was considered desirable to determine the temperature at which it would cease growing in soil. Some sterile soil was mixed with an emulsion of the organism in a sterilized dish, with sterilized spatulas and in an inoculation cabinet. The soil so prepared was transferred in five-gram portions to each of the sterile culture tubes of equal bore through a sterile funnel.

The thermal death point in soil was determined by exposing these soil tubes (in duplicate) to various temperatures. The exposure was for 10 minutes at each temperature and the temperature ranged between 46°C. to 70°C. with an interval of two degrees. The tubes immediately after exposure were immersed in a bath of cold water and transfers made to soil extract mannite agar slants from each.

After making the transfers the exposed tubes were further incubated to see if any chance organism might have been left undetected. The results obtained were as follows :

Strain No.	Lowest temperature (°C.) at which growth was absent
I	54°
II	54°
III	54°
IV	54°

Transfers made after a fortnight's incubation from the soil tubes exposed to temperatures beyond 52°C. did not show any sign of growth on a selective medium.

(*iii*) The author has noticed elsewhere [Madhok, 1934] that the berseem organism from fully grown soil cultures, could survive an exposure for eight hours to the sun-light at a temperature up to 63°C.

With this idea in view, some sterile soil flasks were inoculated with an emulsion of the organism, stirred thoroughly with sterile spatulas and then incubated to get the maximum growth possible. At the end of a week these soil cultures were re-stirred in order to break up any lumps, and five-gram portions transferred to sterile plugged tubes of equal bore.

These tubes were exposed to different temperatures (10 minutes' exposure at each temperature) in a water bath and transfers made to soil extract mannite agar slants after each exposure. The examination of the transfers after four days showed that the organism in these cultures had survived at a temperature as high as 75°C.

Radial movement in soil

Since the nodule organism invades the roots of the host plant only in the "swarmer" or the motile stage, it was considered of interest to find the rate of movement of the berseem organism in soil and thus to get an idea of the spread of the organism from the point of inoculation.

For this purpose a technique similar to that used by Thornton and Gangulee [1926] for the lucerne organism was adopted, but, contrary to their observations, it was found that the berseem organism travelled very slowly in soil.

Observations were made on the soil contained in Petri dishes.

Experimental

(*i*) Ordinary loam soil with optimum moisture content was packed with hand in Petri dishes of about 6-in. diameter. The Petri dishes so packed were sterilized for two and a half hours at a pressure of 20 lbs. On cooling a little hole was made in the centre of the Petri dish by removing the soil with a sterilized cork borer. The hole so made was filled with sterile sand.

The inoculum was given by adding a couple of drops of the suspension of a week old culture on to the sterile sand in the centre, care being taken to add culture suspension just sufficient to moisten the sand up to the periphery.

After the lapse of one, two or four days a boring was taken (in triplicate) at different distances from the centre by means of a sterile cork borer and plated on soil extract mannite agar, with the results given below :

Incubation period (Days)	Distance from the centre		
	0 in.	½ in.	1 in.
1 . . .	+	— — —	— — —
2 . . .	+	— — —	— — —
4 . . .	+	— — —	— — —

+ means growth present.

— means no growth.

This operation was repeated with the same results showing that in a loosely packed soil (unpressed) the organism does not move even half an inch in four days

(*ii*) The above experiment was repeated with the addition of one per cent sugar and the following results were obtained :—

Incubation period (Days)	Distance from the centre		
	0 in.	½ in.	1 in.
1	+	— — —	— — —
9	+	— — —	— — —
14	+	— — —	— — —

Note.—After the expiry of 9 days a shining growth was noticed around the centre about ½ in. in diameter. However, on longer keeping it did not spread any further. It was probably the slime of the organism.

(*iii*) The addition of certain other carbohydrates to soil was also tried and the following results recorded :—

Incubation period	Soil treated with one per cent	Distance from the centre	
		½ in.	1 in.
9 days	Mannite	— — —	..
9 days	Sucrose	— — —	..
20 days	Mannite	+ + +	— — —
20 days	Sucrose	— — —	— — —

(*iv*) To see if the air spaces between the particles of the soil hindered the movement of the organism in soil, hard packing of the soil was tried against loose packing. It gave the following results :

Incubation period	Loose packed		Hard packed	
	½ in.	1 in.	½ in.	1 in.
15 days	— — —	..	— — —	..
20 days	..	— — —	+ + +	+ + +

(*v*) Another variation was made by the addition of 0·1 per cent dipotassium phosphate to the soil, since the compound has been known to increase the motility of the organism. The results were as follows :—

Incubation period	Soil moistened with 0·1 per cent K_2HPO_4		Soil without K_2HPO_4	
	½ in.	1 in.	½ in.	1 in.
10 days	— — —	— — —	— — —	— — —
14 days	+ + +	— — —	— — —	— — —

From the foregoing experiments it is clear that the organism is capable of moving in the soil only at a very slow rate. It spreads in the soil more through the agency of wind and water than by the help of its flagellæ.

Cross inoculations

In order to study the cross inoculation relationship of the organism, twenty-four glass tubes of about 8 in. length and 1½ in. bore were filled about three quarters each with a light loam soil and sterilized for 2½ hours at 20 lbs. pressure. These

were sown with berseem seed sterilized for five minutes in 1/500 mercuric chloride solution, and inoculated in triplicate with an emulsion of the nodule organism cultures isolated from gram (*Cicer arietinum*), *maina* (*Medicago denticulata*) *senji* (*Melilotus parviflora*), *methe* (*Trigonella foenum graecum*), lucerne (*Medicago sativa*), *shaftal* (*Trifolium resupinatum*) or berseem (*Trifolium alexandrinum*). Three tubes were left to serve as controls.

The cultures used in the experiment had been grown on soil extract mannite agar and were incubated at 30°C. for seven days before use. They had been tested for purity and their ability to form nodules on the host plants.

The tubes were kept on a laboratory table in diffused light* and the cotton plugs were removed only when the plants had developed sufficiently high. The roots were examined after about two months and it was found that only the organism of the *shaftal* nodules could inoculate berseem seed.

Test seed	Strain of the organism inoculated with	No. of nodules per plant	Remarks
Berseem	Gram organism	No nodules	
,,	,,	,,	
,,	,,	,,	
Berseem	Maina organism	No nodules	
,,	,,	,,	
,,	,,	,,	
Berseem	Senji organism	No nodules	
,,	,,	,,	
,,	,,	,,	
Berseem	Methe organism	No nodules	Root system very extensive
,,	,,	,,	
,,	,,	,,	
Berseem	Lucerne organism	No nodules	
,,	,,	,,	
,,	,,	,,	
Berseem	Shaftal organism	4, 3, 3, 6 : 4	
,,	,,	7, 2, 2, 1 : 3	
,,	,,	6, 4, 3, 3 : 4	
Berseem	Berseem organism	4, 2, 3, 6 : 4	
,,	,,	3, 6, 8, 10 : 7	
,,	,,	3, 2, 5, 6 : 4	
Berseem	Control	*nil*	Roots very extensive
,,	,,	,,	
,,	,,	,,	

* The column of soil in each tube was kept wrapped up in black paper during the course of the experiment.

In order to ascertain whether the berseem organism could reciprocally inoculate *shaftal*, the experiment was repeated in pots.

Eighteen pots of 4 in. diameter and about 4 in. height were each three parts filled with a light loam soil and sterilized for two hours at 20 lbs. pressure, on each of two consecutive days. Nine of these pots were sown with sterilized berseem seed and the other nine with sterilized *shaftal* seed. Of the nine berseem pots three were inoculated with an emulsion of the berseem organism and three with an emulsion of the *shaftal* organism. Similarly three pots of *shaftal* were inoculated with the *shaftal* organism and three with the berseem organism.

Three pots for each variety of seed tested were left to serve as controls, and were kept on the table in a green-house unexposed to wind or rain. Their roots were examined after two months when the following observations were made :—

Pot No.	Test seed	Inoculated with	Nodulation
1	Berseem	Berseem organism	Plenty
2	,,	,, ,,	,,
3	,,	,, ,,	,,
4	Shaftal	Berseem organism	Plenty
5	,,	,, ,,	,,
6	,,	,, ,,	,,
7	Berseem	Shaftal organism	Plenty
8	,,	,, ,,	,,
9	,,	,, ,,	,,
10	Shaftal	Shaftal organism	Plenty
11	,,	,, ,,	,,
12	,,	,, ,,	,,
13	Berseem	Uninoculated	*nil*
14	,,	,,	,,
15	,,	,,	,,
16	Shaftal	Uninoculated	*nil*
17	,,	,,	,,
18	,,	,,	,,

From the results obtained from these two sets of experiments it is clear that cross inoculations of berseem and *shaftal* are reciprocal.

Effect of ultra violet rays on berseem organism

Ultra violet rays are known to be injurious to bacteria but in an experiment conducted by us in this Laboratory the results [Madhok, 1934] showed that the injurious effect of the sun's rays on cultures of this organism on agar or in soil is

due to the rise in temperature of the medium, rather than to the intensity of the sun's rays in ultra violet radiations.

It was therefore considered necessary to test the effect of pure ultra violet light on agar and soil cultures of this organism. For this purpose a good growth of the organism was obtained on the surface of soil extract mannite agar in Petri dishes. These dishes were exposed in duplicate to a mercury arc lamp for varying durations of time.

The soil cultures were prepared in Erlenmayer flasks by inoculating sterilized soil with an emulsion of the organism. These cultures were also incubated for a week to obtain good growth.

At the time of exposure the soil culture was poured in sterile Petri dishes and exposed in duplicate to the ultra violet rays of the mercury arc lamp for 5, 10, 20 or 30 minutes.

The exposures were made at Lahore 90 miles away and the exposed plates were brought to the Laboratory and platings done as soon as possible. The time that elapsed between exposure and plating was about 9 hours, the temperature during this interval varying between 40° and 45°F.

The counts obtained are given below :—

TABLE II

Showing the effect of ultra violet rays on agar and soil cultures

No.	Time of exposure	Dilution	Average count	Total count
		Agar cultures		
1	Control . . .	1 million	4,200	4,200,000,000
2	5 Mts. . . .	40,000	286	11,440,000
3	10 ,, . . .	40,000	95	3,800,000
4	20 ,, . . .	40,000	65	2,600,000
5	30 ,, . . .	40,000	50	2,000,000
		Soil cultures		
1	Control . . .	100 million	51	5,100,000,000
2	5 Mts. . . .	10 ,,	02·5	4,025,000,000
3	10 ,, . . .	1 ,,	1600	1,600,000,000
4	20 ,, . . .	1 ,,	..	Innumerable
5	30 ,, . . .	1 ,,	..	Do.

The above results tend to indicate that the ultra violet rays have not been able to bring about the death of all the organisms either on the agar surface or in soil cultures. This is clear, however, that the effect of these rays is very injurious, varying directly with the time of the exposure.

The injury is much less serious in soil culture.

VIABILITY OF THE ORGANISM ON THE SEED

(*i*) In order to find out the extent to which the organism could resist desiccation, a quantity of the seed was inoculated and kept stored in a Petri dish in one of the laboratory cupboards.

Sowings were done in triplicate from week to week in sterilized soil pots from this seed. The pots so sown were kept in one of the window spaces away from the direct action of rain, etc. Three control pots were also sown at each interval with sterilized seed. The pots were occasionally watered with sterile tap water. The plants were pulled out after a couple of months and examined for nodule formation.

The results are tabulated below.

TABLE III

Showing the effect of age on the infective power of an inoculated seed

(Seed inoculated on 4th Nov. 1930)

Sown on	Inoculated nodulation	Control nodulation
4th Nov. 1930 . . .	+ + n.g. . .	— — —
10th Nov. 1930 . . .	+ + n.g. . .	— — —
17th Nov. 1930 . . .	+ + + . .	— — —
24th Nov. 1930 . . .	n.g. n.g. n.g. . .	— — —
1st Dec. 1930 . . .	+ + + . .	— — —
8th Dec. 1930 . . .	+ + + . .	— — —
16th Dec. 1930 . . .	+ + + . .	— — —
23rd Dec. 1930 . . .	+ — — .	— — —
30th Dec. 1930 . . .	— — — . .	— — —

n.g.=No germination.

It is clear from the above that the organism retained its infective power on the seed for about 7 weeks only.

(*ii*) Another experiment was undertaken in the same connection. Different portions of seed were inoculated at an interval of a month and stored in sterile Petri dishes in the laboratory cupboard. Three sterile soil pots were sown from each of these portions on the same day and the nodule formation observed after about a month with the following results :—

Date of sowing	Seed inoculated on	Nodulation
28th Nov. 1931 . . .	11th July 1931 . .	— — —
Do. . . .	7th Aug. 1931 . .	— — —
Do. . . .	7th Sept. 1931 . .	— — —
Do. . . .	8th Oct. 1931 . .	+ + +
Do. . . .	Control . .	— — —

This again shows that the organism retains its infective power on the seed for not more than seven weeks.

Conclusions

In the foregoing experiments some of the cultural characteristics of the organism causing nodules on the roots of berseem (Egyptian clover) have been studied. This organism has been found to be a pseudomonas having its cross inoculant in the organism infecting the roots of *shaftal* (*Trifolium resupinatum*).

According to Löhnis and Hansen [1921] who have divided the bacteria of the leguminous plants into two groups, the representatives of which differ both morphologically and physiologically, the organism infecting the roots of berseem (*Trifolium alexandrinum*) should be peritrichic but inspite of repeated trials we have found this organism to possess only a single polar flagellum.

Cultural characteristics of the organism have been studied on a number of different media. The observations recorded for a change in milk, growth on potato and growth on beef extract media agree with those recorded by Löhnis and Hansen [1921] for the organisms of the clover group.

The pH tolerance of the organism has been found as low as 5.0 on the acid side and 10 or probably a little more on the alkaline side. This agrees with the critical pH limits, recorded by Fred and Davenport [1918].

The thermal death point of the various strains of the organism studied, varied from 52°C. to 54°C. The reason why it can withstand the high summer temperatures of the Punjab (48°C.) is probably due to its being protected in the deeper layers of the soil.

As for the claims made by Thornton and Gangulee [1926] that the nodule organism moves about an inch in 24 hours, the same have not been substantiated by us, in respect of this organism.

Its movement in moist soil has not exceeded half an inch in about a fortnight and that even in a hard pressed soil. This leads one to the conclusion that the organism is transferred in soil more through wind and water than through its organs of locomotion.

The effect of ultra violet rays was studied. The ultra violet light did exercise a destructive effect but this effect varied with the time of exposure. Sun's rays which are so rich in these radiations, owing to the non-permeability of the soil particles to these rays, could have only a partially destructive effect on this organism in soil.

As for the viability of the organism on a seed, it has been found that it retains infective power on the seed for not more than seven weeks.

These results do not agree with those of Fellers [1919] who has found out that the soybean and alfalfa bacteria retain their viability for six to nine months. But his method of storage of the inoculated seed was different from ours.

Fellers [1919] has further mentioned that the largest decrease in the number of organisms on a seed-coat occurs within the first few hours. In view of the fact that there is an exceedingly small percentage of the cells (sometime 1 : 1,000,000) present on a seed that are capable of forming nodules, our results are not extraordinary. The organism investigated is probably more sensitive to desiccation than others.

Acknowledgements

The author wishes to express his thanks to S. Ram Singh Sarkaria, the late Agricultural Bacteriologist, for certain suggestions during the progress of the work and to Dr. P. E. Lander, Agricultural Chemist to Government Punjab, for his kind interest and helpful criticism.

Summary

1. The organism producing nodules on the roots of berseem plants is 1 to 3 μ in length and about a μ broad, varying in size at different stages in the life cycle.

2. The organism is mono-trichic with a single polar flagellum.

3. A study of the cultural characteristics of the organism has been made on some of the more important media. The pH tolerance of the organism has been found to be 5 on the acid side and 9·8 or a little more on the alkaline side.

4. The movement of the organism in soil is slow, hardly half an inch in a fortnight.

5. It cross-inoculates with an organism infecting the roots of *shaftal* (*Trifolium resupinatum*).

6. The thermal death point of the organism is between 52°C. and 54°C. But it has been found to survive a temperature of 75°C. in fully grown soil cultures.

7. The organism is sensitive to ultra violet rays. The destructive action of the rays varies with the time of exposure.

8. The organism retains its infective power on the seed, when stored in Petri dishes, for only about seven weeks.

REFERENCES

Albrecht, W. A. (1920). *Soil Sci.* **9**, 275-329.

Bialosuknia, W., and Klott, C. (1923). *Roczn Nauk Rolniczych* **9**, 288-335.

Burrill, T. J. and Hansen, R. (1917). *Ill. Agric. Exp. Stn. Bul.* **202**, 115-181.

Fellers, C. R. (1919). *Soil Sci.* **7**, 217-232.

Fred, E. B. and Davenport, A. (1918). *J. Agri. Res.* **14**, 317-336.

Hansen, R. (1921). *Scientific Agric.* (*Canada*) **1**, 59-62.

Joshi, N. V. (1920). *Mem. Dept. Agri. Ind., Bact. Ser.* **1**, No. 9, 249.

Klimmer, M. and Kruger, R. (1914). *Centbl. Bakt. Abt.* 2, **40**, 256-265.

Löhnis, F. and Hansen, R. (1921). *J. Agric. Res.* **20**, 543-556.

Madhok, M. R. (1934). *Agric. and Livestock in Ind.* **4**, 670.

Nobbe, F., Hiltner, L. and Schmid, E. (1895). *Landw. Vers. Stn.* **45**, 1-27.

Sarkaria, Ram Singh (1933). *Agric. and Livestock in Ind.* **3**, 16-32.

Thornton, H. G. and Gangulee, N. (1926). *Proc. Roy. Soc. Series, B.* **99**, 427-438.

Waksman, S. A. (1926). ' Principles of soil Microbiology ', P. 137.

Wilson, J. K. (1917). *Cornell Univ. Exp. Stn. Bul.* **386**.

Wright, W. H. (1925). *Soil Sci.* **20**, 95-120.

ABSTRACTS

The method of choosing initial material in breeding (533 : 575 : 578. 08 : 581. 143·26·03). I. A. KOSTJUCENKO (*Semenovodstvo—Seed Growing* 1934 : No. 5 : 18 –21).

[*Full summary prepared by the Imperial Bureau of Plant Genetics, Cambridge : Reproduced by kind permission of the Director of the Bureau.*]

In making use of the world collection of varieties for crossing with the local strains the entire success depends on the choice of the most suitable parental varieties. In the past considerable losses of time have been caused by working with unsuitable parental material. Nilsson-Ehle has shewn that to assess the value of varieties it is necessary to grow them under conditions in which they can complete their vegetative cycle. This can be done in Russia only by the method of vernalization. Lyssenko recommends that the best local varieties should be crossed with those world varieties which in the region in question prove earliest after vernalization.

Experiments are described in which various spring wheats were sown in the vernalized and unvernalized state both at Hibiny, with its continuous day, and at Leningrad with its 18-hour day. The cold treatment made hardly any difference to the vegetative period but the long day accelerated it by 3-5 days. This was accompanied by an increase of 0·6-6·6 g. in thousand corn weight which is thus attributed to the effect of the long day in accelerating the second developmental stage, the photostage.

It is necessary therefore to test the parental varieties of long-day plants under continuous illumination to obtain a true picture of their relative merits. For southern regions they will be tested under reduced length of day.

Different plants of a single variety have shewn different reactions to low temperature. Various varieties of winter wheat were vernalized at a temperature of +2°C. for 45, 35, 25 and 20 days. Controls were sown at the same time as the experimental plants. Only 12 of the 60 varieties tested shewed clear response to the cold treatment ; some of these moreover reacted only in the case of treatment for 45 days, others only to the 45 and 35 days treatments, certain others again only to 45, 35 and 25 days. In these two latter groups both winter and semi-winter types were detectable amid the plants which came into ear normally. Similarly again on sowing certain varieties in spring, especially certain Chinese and Japanese varieties, it was observed that some plants came into ear normally whilst others behaved as winter or semi-winter forms, whereas after vernalization treatment these same varieties eared and matured normally and uniformly.

It is clear therefore that many lines which morphologically are pure, with regard to their developmental stages are definitely heterozygous. This heterozygosity is regarded as being partly responsible for the irregular segregations often observed in characters such as yield, earliness, etc. In any case it shews the necessity, in choosing parental forms, of basing conclusions only on the progeny of single plants and not on plants chosen at random from a particular line or variety.

Catalogue number	Origin and name of variety	Variety	Vegetative period from emergence to earing						Reduction in vegetative period in days as a result of increased length of day in the second developmental stage	Thousand corn weight						Increase in thousand corn weight as a result of increased length of day in the second developmental stage
			Vernalized			Control				Leningrad (18-hour day)			Hibiny (24-hour day)			
			Leningrad (18-hour day)	Hibiny (24-hour day)	Difference	Leningrad (18-hour day)	Hibiny (24-hour day)	Difference		Vernalized	Control	Difference	Vernalized	Control	Difference	
...	U. S. S. R. 'Novinka'	erythrospermum	42	33	9	42	34	8	8	18.3	18.9	0.6	39.1	36.8	2.3	2.3
25725	India, Pusa 52	grecium	42	33	9	42	33	9	9	18.4	18.1	0.3	40.3	39.2	0.9	0.6
21105	Asia Minor	Reichenbachei	42	35	7	43	35	8	7	16.3	15.3	1.0	37.4	32.6	4.8	3.8
25626	South Africa	albidum	39	33	6	38	33	5	5	22.0	20.5	1.5	47.7	39.6	8.1	6.6
8085	Canada 'Garnet'	lutescens	39	33	6	39	33	6	6	17.6	17.3	0.3	27.7	32.6	5.1	4.8
25550	Australia	alborubrum	42	34	8	40	34	6	6	17.2	20.7	—3.5	36.5	34.5	2.0	2.0
...	Moscow 2411	erythrospermum	47	41	6	winter	winter	0	6	19.2	...	...	...	...	...	...
14925	U. S. A. 'Nebraska'	erythrospermum	48	41	7	,,	,,	0	7	20.4	...	...	...	...	...	...

Effect of long day in reducing the vegetative period and increasing the grain weight.

The after harvest ripening of winter wheat in northern regions (633·11 : 581·142) L. TRET' JAKOVA (*Semenovodstvo—Seed Growing* 1934 : No. 6, 33—36).

[*Full Summary prepared by the Imperial Bureau of Plant Genetics, Cambridge : Reproduced by kind permission of the Director of the Bureau.*]

The germinating capacity of the seeds of a number of varieties of winter wheat was tested at varying periods after harvest, from 5 to 50 days. Marked varietal differences were observed, for instance the rye-wheat hybrid and the variety *ferrugineum* 2411 of the Moscow station gave 60-70 per cent germination on the 5th day, rising to 90 per cent on the 35th. Durable and Ukrainka gave a much lower germination on the 5th day and took much longer to reach full germinating capacity—this was attained in the former only on the 45th and in the latter on the 50th day. The variety *erythrospermum* 917 was intermediate between these two types, as were all the other varieties tested.

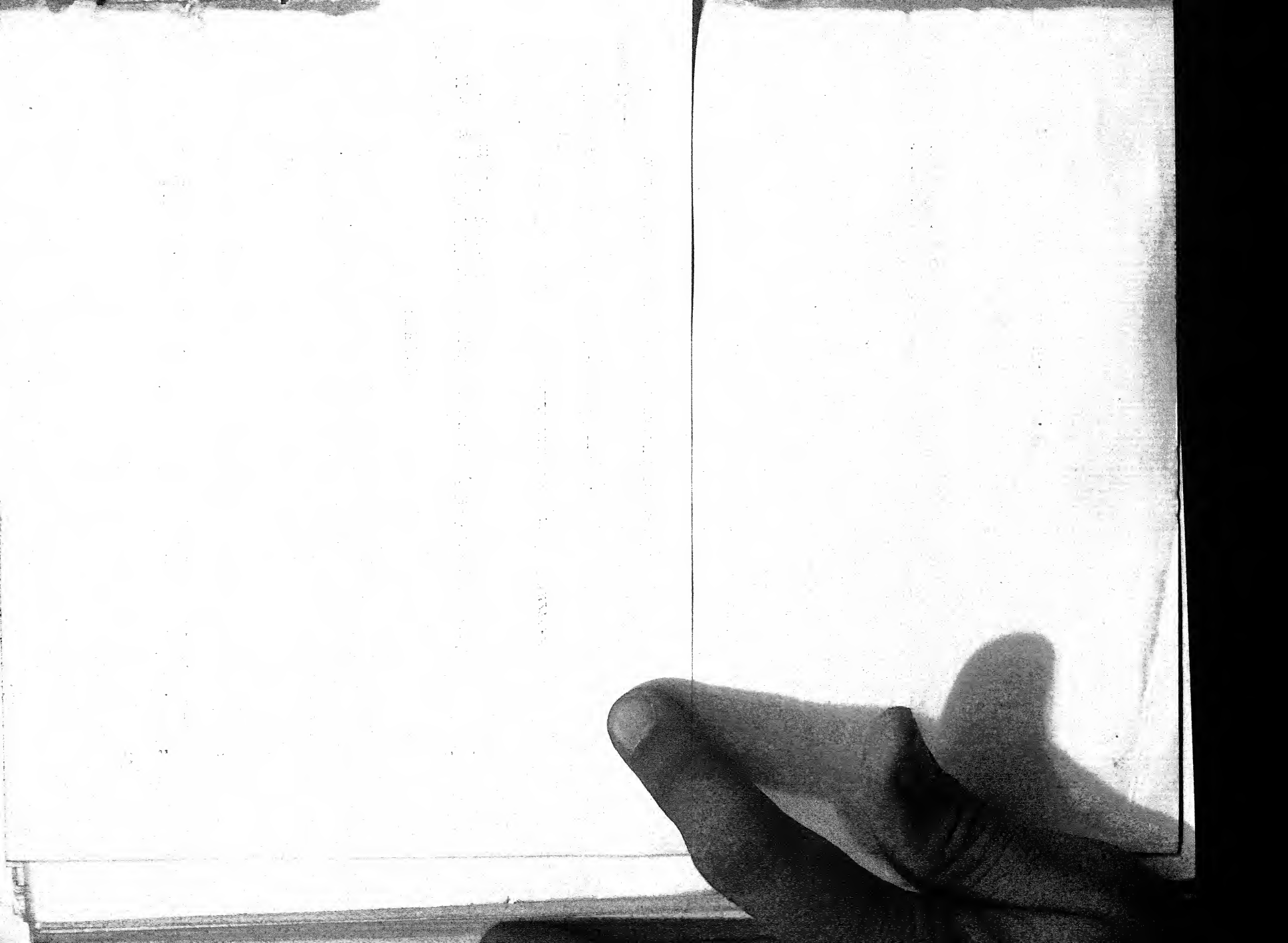
At another station the rye-wheat hybrid and *ferrugineum* 2411 attained full germinating capacity on the 23rd day whereas Ukrainka was nearly three weeks later. In many localities the date of sowing is past before the majority of the varieties have attained their full germinating capacity.

To study the relation between germination and moisture, the grain of a number of varieties was harvested at different stages of maturity, beginning at the wax-ripe stage, at which all varieties displayed a high germination percentage, which in the rye-wheat hybrid was as much as 98 per cent. Three days later, towards the end of the wax-ripe stage and the beginning of the stage of full maturity, a marked diminution in moisture content was observed in all varieties, falling in some to 10 per cent. At this stage also the rye-wheat hybrid gave the highest germination, though it was lower than at the earlier stage. The changes in germination capacity when compared with those of moisture content were found to be independent. In the stage of complete maturity there is a marked reduction in germination, with only a very small change in moisture content. Later there occurs a parallel rise in germination capacity and fall in moisture content, but even still when all varieties had reached the same moisture content the differences in germination remained quite marked.

Thus some varieties reach a high germinating capacity while retaining a considerable proportion of moisture, whilst others may have a high germination combined with a normal moisture content at a time when their process of maturation is yet incomplete.

The seeds were germinated at 18°C. Germination in river sand invariably gave a higher germination than on filter paper. Chilling for three days at a temperature of 10-13°C. induced rapid germination in unripe seeds which unchilled germinated only very slowly.

GIPD—S 2—35 ICofAR—15-7-35—800.



ORIGINAL ARTICLES

HELMINTHOSPORIUM DISEASES OF BARLEY AND THEIR CONTROL

BY

M. MITRA, M.Sc., PH.D., D.I.C.,

Assistant Mycologist, Imperial Institute of Agricultural Research, Pusa,

AND

R. D. BOSE, B.Sc.,

Offg. Assistant Economic Botanist, Imperial Institute of Agricultural Research, Pusa

(Received for publication on 7th February 1935)
(With Plates XVIII and XIX and four text-figures)

CONTENTS

I. Introduction

Three species of *Helminthosporium* are generally responsible for diseases of barley. In the seedling stage many plants succumb to the foot and root-rot diseases which these fungi cause and in the mature crop these fungi are responsible for blotches and spots on leaves, thus lowering photosynthetic activity of the plants by reducing the leaf area. In the barley growing tracts of Northern India, *H. sativum* P. K. & B., which causes a foot and root-rot disease in the seedling stage and leaf blotches later, is the more common pathogen. *H. teres* Sacc. has also been found to attack some types of barley. *H. gramineum* Rabh., which causes the severe stripe disease in barley in Europe and Northern America, is less common on the whole, though in the United Provinces of Agra and Oudh, it causes some damage.

H. sativum has, in recent years, been found in an epidemic form causing severe foot and root-rots at Pusa and also the blotch disease, leading to reduction in crop yields. The percentage of seedlings killed and the leaf area destroyed in mature plants have shown a good deal of variation in different types of Pusa barleys as well as in other Indian and foreign types grown at Pusa. The variation has not only been from type to type but also from season to season in the same type. The degree of attack has also varied from one locality to another, from plot to plot and sometimes also in different parts of the same plot. It has also been noted that some types of Pusa barleys are resistant to a certain extent, while others have shown high susceptibility, though none of them have been completely immune. The present investigations were undertaken in order to determine the varietal resistance of the Pusa types of barley to this species of *Helminthosporium* and the effect of certain fungicides in controlling the foot-rot and root-rot phases of the disease, and the data collected during the past four years are discussed in this paper.

II. Symptoms

These three *Helminthosporium* species cause certain definite and distinct symptoms in the barley plants.

(*i*) *Spot blotch*

H. sativum is the most destructive of the three species of *Helminthosporium* and it causes the foot and root-rot, the seedling blight, the leaf-spot, the head blight and the discoloration of seed (Plate XVIII). It stunts the growth of the barley plant, retards the development of roots and thus weakens its standing power. Even seed germination is lowered and finally causes appreciable loss in yield. The disease thus is manifested in two phases :—

(*a*) Primary infection due to the infection from the seed causing foot-rot and root-rot of seedling.

PLATE XVIII

HELMINTHOSPORIUM SATIVUM

Six seedlings of barley showing various stages of 'foot-rot' and 'root-rot.' Leaves show spot formation (natural size).

(*b*) Secondary infection due to the conidia borne on the primary spots and causing fairly well defined, more or less longitudinal spot formation on all aerial parts on account of which it is called ' spot blotch '.

(*a*) *Primary infection* (*Foot-rot and root-rot*).—The first symptom of the disease in the field becomes apparent during the seedling stage of the barley plant. The seedlings show the appearance of blight very much like damping off. When severely attacked, the plants are killed at the ground level. A large number of diseased seedlings fail even to come out of the soil and the roots and the young shoots of such diseased seedlings show deep brownish discoloration, become brittle, and rot. The injury to the root is brought about by a rather limited number of local lesions which kill the root tips. When these lesions occur back from the tip they some times cut off portions of the root as well. Very often the emerging roots get infected and rot. In other cases the root system is fairly well developed and may be free from the disease or may be slightly attacked, but the stems show brownish discoloration at the foot and are soon destroyed. Sometimes the seedlings produce a few leaves but as the roots are attacked by the fungus the plants soon die. Severely infected seeds do not germinate but if they do germinate the seedlings generally do not emerge from the ground level, or sometimes only the tips of the leaves push out before the seedlings are killed. Infected plants are of course distinctly stunted. When such plants are up-rooted they show poor development of the root system, which has a brownish discoloration in parts due to the attack of the fungus. Very often the roots become so brittle and decayed that an attempt to pull them up causes them to break at the crown.

(*b*) *Secondary infection*.—The secondary phase of the disease is manifested by the appearance on the leaf blade of spots varying from 0·5 to 15 mm. in width and from 2 to 30 mm. in length. The blotches are oval or irregular, usually brown to dark brown and the colour fades at the margin of these blotches in the form of yellow zones which gradually merge into the green of the surrounding tissue. The coalescence of several spots results in the formation of big irregular spots. Often, as the fungus progresses into the leaf tissue, the spots become darker and finally reddish in colour, forming short, narrow, dark brown streaks. Blotches appear on the leaves of the seedlings as well as on those of the grown-up plants. The lower leaf blades are first affected and the blotches multiply until scores of them appear on one foliar organ and a considerable portion of the leaf tissue becomes involved. The entire blades are often affected to such an extent that they curl up and cause the death of the plant.

The fungus also forms spots in the stem especially at the nodes, attacks the inflorescence and may be found on glumes and not infrequently on the seed, sometimes causing the entire head or portion of it to be blighted. The diseased kernels usually are readily distinguished because of the dark brown discoloration at the germ end and such seeds when placed in a moist chamber give rise to a woolly mycelial growth within a day or two. Such severely affected seed occurs in years

when the disease is in an epidemic form and shows great reduction in the percentage of seed germination. In the case of very severe attacks the ovary is infected and under such conditions no seed is formed; whatever seed is set is badly shrunken and incapable of normal germination.

On the plots in which barley is grown year after year the disease is worst during the seedling stage and *H. sativum* has been isolated from such soil samples. This shows that the organism may live in the soil as well as in and upon infected barley grains. When the diseased kernels are planted, the fungus becomes active upon the germination of the seed, penetrates into the young root and leaves and causes reddish-brown to dark-brown spots.

(*ii*) *Net blotch*

The disease caused by *H. teres* is recognised by the appearance on the leaf blade of dark brown spots or streaks which at first are about 1 mm. in diameter but which, later, increase considerably in size in a longitudinal direction. The most distinctive feature of the species is the discoloured area involved in the net-blotch lesions. This characteristic "netting" of the discoloured area on leaves, sheaths, glumes, spikelets and grains distinguishes this blotch from the spot-blotch disease. Further, it does not cause splitting of the leaves which is so common in the stripe disease. The straw at harvest is dull brown and lacks strength. This disease does some damage to barley in India, and is restricted to only a few types and does not occur on local or on other exotic barley types grown at Pusa. The net-blotch disease, is common in the United Provinces and in Nepal but no report of any serious damage has hitherto been recorded.

This fungus is also known to cause foot-rot and root-rot in other countries but it has never been noticed at Pusa.

(*iii*) *Stripe disease*

The disease caused by *H. gramineum* is characterised by the occurrence of straw-coloured longitudinal streaks on the leaf blades, the typical stripe of the lesions. Another marked symptom of this disease is the failure of the ear to set seed normally. The disease first makes its appearance in the form of small yellowish spots on the leaves, later extending into long parallel streaks. The streaks become dark-brown or reddish brown and the whole leaf gradually dries up. The leaf blades are attacked as they develop, and sometimes the ear does not emerge from the sheath; if it does, it remains erect and stunted and the awns often become twisted and bent. The affected heads turn greyish-brown and the grains within are usually shrivelled. As the diseased portion of the blade within the yellowish green stripes dies, a splitting or shredding of the entire blade occurs. Dead leaves and plants soon crinkle and ultimately die. Fortunately, this serious disease of barley is not very common in India.

III. Host range and strains of *H. sativum*

H. sativum is not restricted to barley and is vigorously parasitic on several grasses, according to Stevens [1922] and Christensen [1926]. It has also been found to form spots on paddy and maize at Pusa and has been successfully inoculated in India on wheat, oats, sorghum, sugarcane, *Setaria italica and Panicum frumentaceum* by Mitra [1931, 1]. On all these hosts this organism is found to produce distinct spots on which fructifications are formed. The longevity of spores of *H. sativum* may extend to at least four years under Indian conditions. The organism can live as a facultative saprophyte on dead and dying plants. Its greatest luxuriance, however, is attained on the foliage of wheat and barley. There are several strains of this fungus but the most common are those on barley and wheat. The wheat strain is as vigorous as the barley one and both the strains are capable of infecting either of the two hosts.

Mitra [1931, 2] obtained several saltants of this fungus in culture media and some of these were later isolated from the host plants. This demonstrates that the saltants which arise in culture also occur under natural conditions. A study of the saltants of these two strains from wheat and barley has established the fact that the barley strain is capable of giving rise to saltants of the wheat strain type and *vice versa* [Mitra ; 1931, 2].

IV. Temperature relationship of *H. sativum*

Detailed studies in the morphological characters of the barley and wheat isolates of *H. sativum* have been recorded by the senior author [Mitra ; 1931, 2]. The rate of growth of the two strains at different temperatures has also been studied on different media and the general relation between temperature and the linear growth is shown in Figs. 1 to 4. Figs. 1 and 2 give the rate of spread of the barley strain on Brown's synthetic agar and Brown's synthetic starch agar over series of temperatures. Figs. 3 and 4 give a comparative picture of both barley and wheat strains on three media.

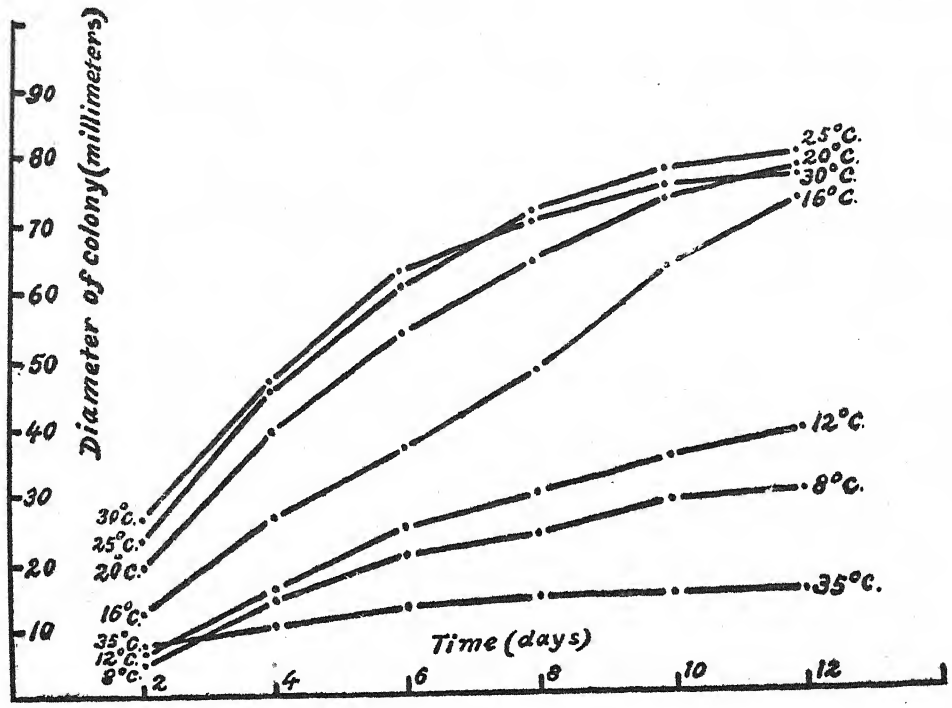


Fig. 1. Temperature relationship of *H. sativum* P. K. & B. (barley) on Brown's synthetic agar

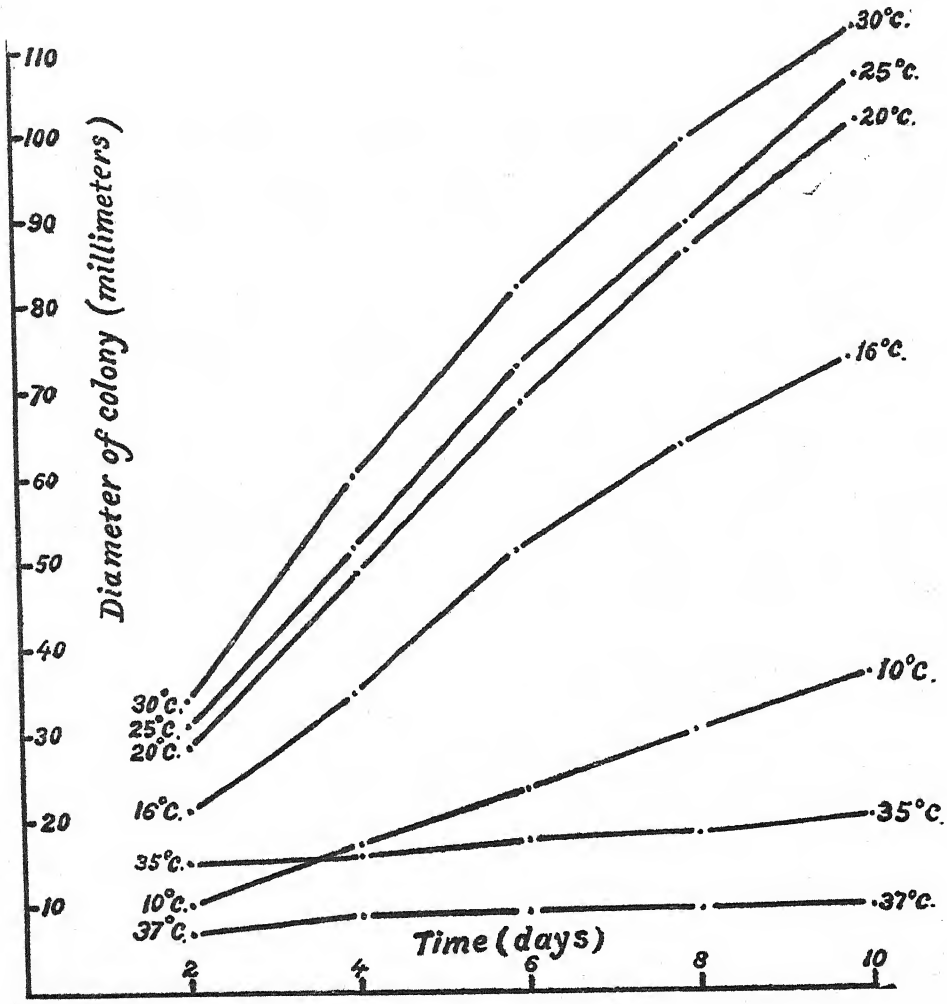


Fig. 2. Temperature relationship of *H. sativum* P. K. & B. (barley) on Brown's synthetic starch agar

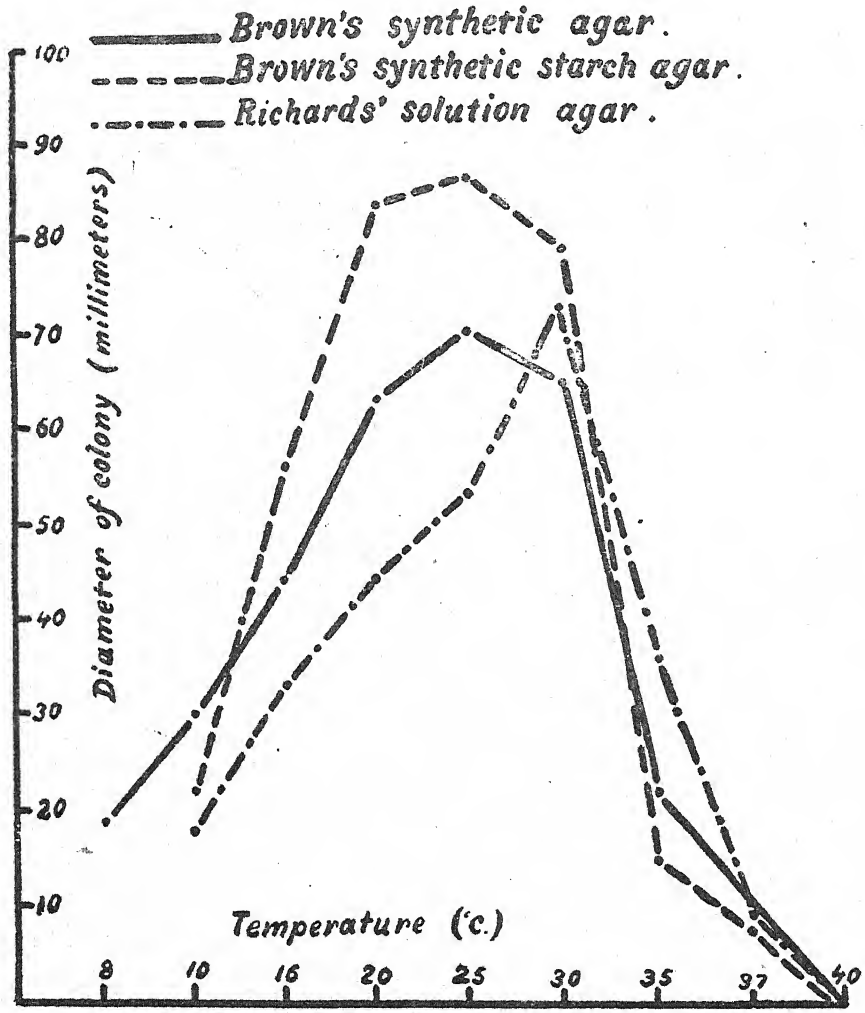


Fig. 3. Eight days' growth of *H. sativum* (wheat) on Richards' solution agar at various temperatures

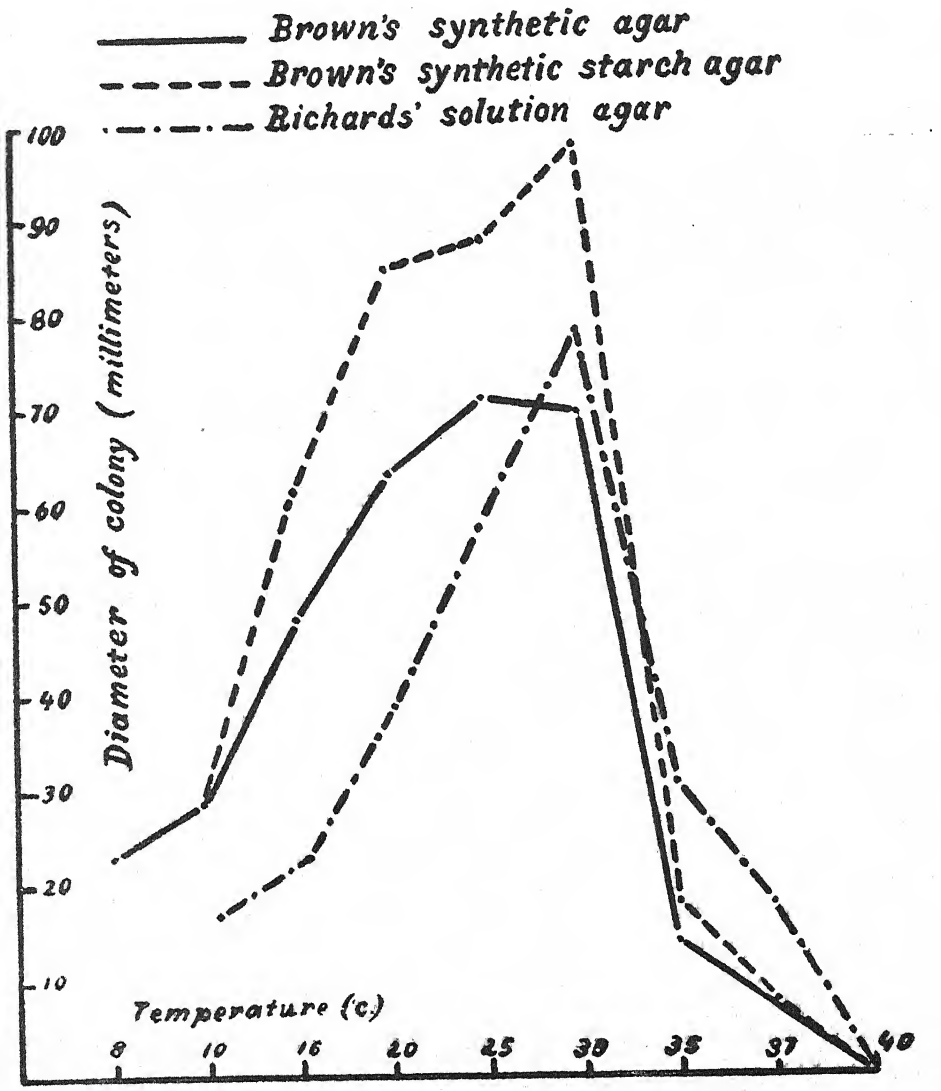


Fig. 4. Eight days' growth of *H. sativum* (barley) on Richards' solution agar at various temperatures

The optimum temperature for the two strains of *H. sativum* on different media is :—

TABLE I

Strain	Brown's agar	Brown's starch agar	Richards' agar
Wheat . . .	25°C.	25°C.	30°C.
Barley . . .	25°C.	25-30°C.	30°C.

From these figures we conclude that the optimum temperature for the growth of *H. sativum* lies between 25 and 30°C. Dosdall [1923] in her studies with the American strains of this fungus found the optimum between 24 and 28°C.

V. THE METHOD EMPLOYED IN DETERMINING THE PERCENTAGE OF LEAF AREA DESTROYED

In preliminary studies the amount of damage done to the leaf area was obtained by using comparative but arbitrary terms such as very slight, slight, fair and bad to represent the degrees of attack. These standards, however, are liable to alteration from season to season and from observer to observer. Personal errors thus crept in and a correct estimate could hardly be obtained. In order to overcome this difficulty a standard chart was prepared following Tehon [1927], in which leaf blades with 2, 5, 10, 15, 20, and 30 per cent area destroyed by the fungus, were diagrammatically represented. Such a chart is shown in Plate XIX.

The percentage of the leaf area destroyed in any type of barley was estimated by collecting the second and fourth leaves from a number of random plants in that type and then classifying the leaves according to the standard chart. A frequency distribution was thus obtained and the average value calculated as shown in Table II.

PLATE XIX.

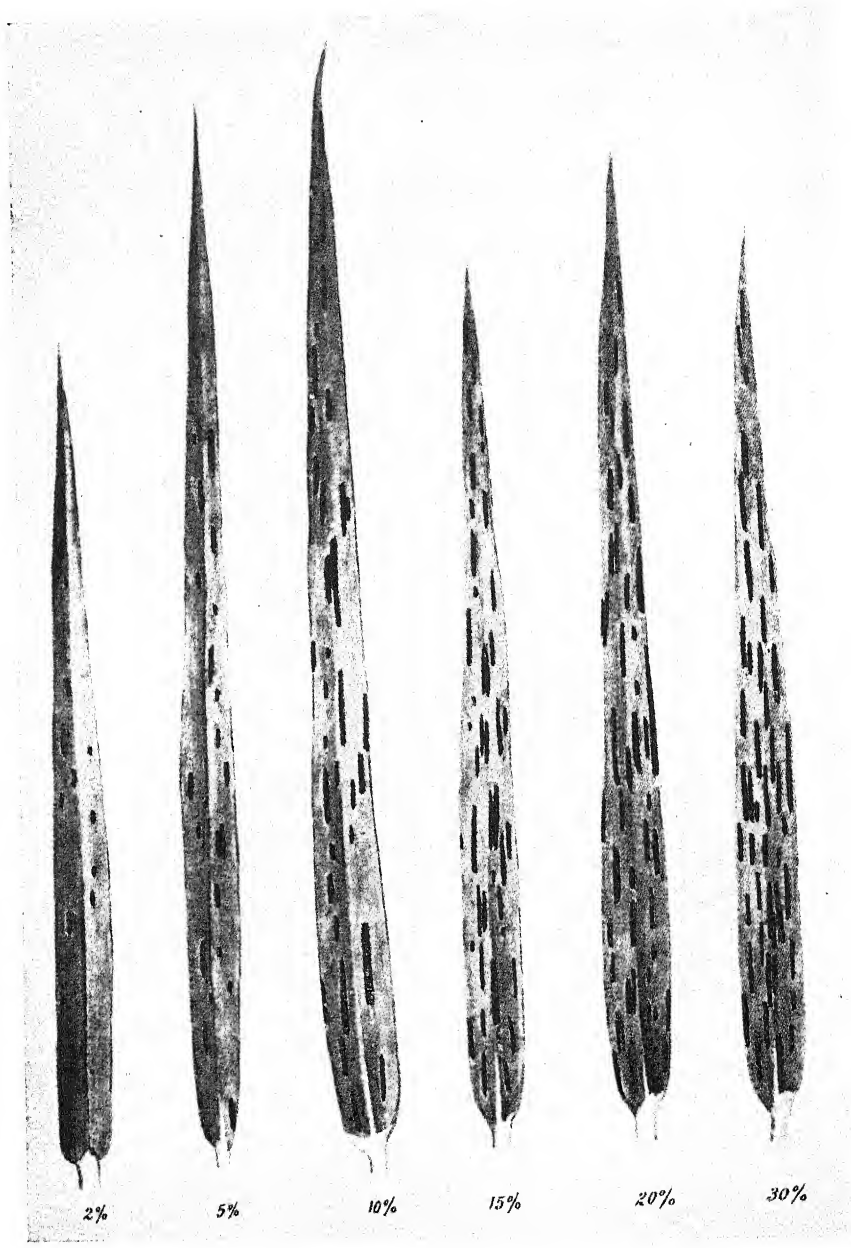


Chart for determining the percentage of leaf area destroyed.

TABLE II

Distribution of leaves of barley type 21 according to the percentage of leaf area destroyed

Seed treatment before sowing	Replication	Percentage of leaf area destroyed							Total no. of leaves	Average damage
		0	2	5	10	15	20	30		
Untreated—control	1	23	33	5	..	..	..	..	61	1.4
	2	38	38	3	..	..	..	..	79	1·1
	3	26	40	5	..	..	..	..	71	1·4
	4	20	35	7	3	..	..	..	65	2·0
	5	24	40	2	..	..	..	..	66	1·3
	6	30	39	6	1	..	..	..	76	1·5
	7	15	20	16	1	..	..	..	52	2·5
TOTAL .	..	176	245	44	5	..	..	..	470	Mean 1·6170 ±0·0952
Disinfected with uspulun (universal)	1	46	7	..	..	..	..	..	53	0·26
	2	103	..	..	..	..	..	..	103	0·00
	3	97	..	..	..	..	..	..	97	0·00
	4	76	..	..	..	..	..	..	76	0·00
	5	126	..	..	..	..	..	..	126	0·00
	6	107	2	..	..	..	..	..	109	0·03
	7	37	1	..	..	..	..	..	38	0·05
TOTAL .	..	592	10	..	..	..	..	..	602	Mean 0·0332 ±0·0040

Mean difference = 1.5838 ± 0·0953

The data presented as an illustrative example in the above table have reference to the method of treatment which is to be hereafter explained and have been cited here to show how average leaf areas destroyed are to be calculated.

The mean difference of 1·5838 ± 0·0953 per cent is statistically significant and denotes that a definite advantage has been gained in reducing the extent of the disease by disinfecting the seed with uspulun before sowing. This method of determining the leaf area destroyed by species of *Helminthosporium* affords a reliable estimate and is amenable to comparisons being made between different types in the same or in different seasons.

VI. Effect of environmental factors on disease development

Observations made during the last four years indicate that *H. sativum* is present every year in practically every field of barley at Pusa and that it is more destructive in some years than in others. Table III shows the percentage of leaf-area destroyed by *H. sativum* and *H. teres* in twenty-four types of barley [Bose, 1931] and the local country type during 1930-31, 1931-32, 1932-33 and 1933-34, and brings out the fact that there is a wide variation in the virulence of the disease on different types of barley from season to season. It will also be noticed that *H. sativum* attacks most of the Pusa barley types, whereas *H. teres* attacks only certain types which have been selected from samples originally collected from different parts of India [Bose, 1931]. The local type usually grown in the neighbourhood of Pusa is less susceptible to *H. teres*. In addition to the data presented in Table III the writers have observed the disease due to *H. sativum* in several areas in the neighbourhood of Pusa and have noted great variation in the incidence of the disease from year to year, from plot to plot and even in different parts of the same plot. This perhaps is due to differences in soil moisture, temperature, etc. Thus, it is apparent that certain ecological factors influence profoundly the severity of the disease. The fungus manifestly enters but remains quiescent in the host tissues when conditions are unfavourable preventing the pathogen from becoming destructive. Under favourable conditions, however, secondary infection may occur repeatedly till harvest.

Observations show that heavy dew and rain together with high temperature are conducive to foliage and spike infections. Infection can take place at any time of the season. The fungus has a wide range of temperature relationship and the spores are capable of germination even at 8°C. or at 33-35°C., the optimum temperature being between 25 and 30°C., that is, nearabout 28°C. As already shown by the senior author [Mitra ; 1931, 1] the higher temperatures are most favourable for the growth of the fungus, for spore germination and infection and generally for the development of the disease. He found that the amount of infection considerably increases as the temperature is raised from 20°C. to 30°C. The fungus can remain dormant in the host tissue for a long time under conditions unfavourable for its spread. At the time barley is sown at Pusa, the mean atmospheric and soil temperatures are nearer the optimal limits of the pathogen and hence considerable damage is caused during the seedling stage. The mean temperatures then fall and the fungus is inactive during the winter months, but with the approach of the spring at the time of heading out the atmospheric temperature again rises and the disease increases causing a good deal of secondary infection. The full effect of the destructive nature of the fungus becomes apparent when the crop is mature and is about to be harvested. Early varieties naturally escape the disease due to the comparatively lower temperatures prevailing at the time they mature.

It has been noticed that *H. gramineum* is either absent or very rare at Pusa and in its vicinity, and occurs only during certain years when a foreign type is grown at Pusa, *e.g.*, ' Cape barley' grown during 1921 in the Farm was very severel attacked by ' stripe disease ' and the whole crop had to be burnt. This rare occurrence of ' stripe disease ' is possibly due to the fact that temperatures at Pusa are not suitable for the growth of this organism. Johnson [1925] found that low soil temperature favours infection of barley by *H. gramineum* and that very little ' stripe disease ' occurs at temperatures higher than 20°C. Such low temperature does not occur at Pusa either at the time of sowing or harvesting which may account for the absence of the disease from Pusa.

VII. Epidemiology of the disease during 1930-34 on various types of barley

The percentage of leaf area rendered ineffective by *H. sativum* and *H. teres* on barley varieties grown at Pusa from 1930 to 1934 is shown in Table III. These percentages are based on an estimation from about 200 leaves in each type.

Table III

The percentage of leaf area rendered ineffective by Helminthosporium teres *and* H. sativum *on 24 types of barley during 1930-34*

Type	1930-31			1931-32			1932-33			1933-34		
	Attacked by		Combined infection	Attacked by		Combined infection	Attacked by		Combined infection	Attacked by		Combined infection
	H. teres	*H. sativum*		*H. teres*	*H. sativum*		*H. teres*	*H. sativum*		*H. teres*	*H. sativum*	
1	1.8	4.1	5.9	1.0	2.6	3.6	...	0.26	0.26	...	4.6	4.6
2	...	3.0	3.0	...	1.2	1.2	...	1.3	1.3	...	13.1	13.1
3	1·2	1.2	2.4	...	1.4	1.4	...	2.6	2.6	...	16.5	16.5
4	6·3	...	6.3	...	5.2	5.2	0.71	2.14	2.85	...	14.8	14.8
5	12·1	...	12.1	1.0	3.9	4.9	0.81	2.44	3.25	1.1	11.2	12.3
6	27·9	...	27.9	23.2	...	23.2	20.45	...	20.45	20.7	3.2	23.9
7	21·2	...	21.2	25.4	...	25.4	14.4	...	14.4	10.1	1.1	11.2
8	10·6	4.5	15.1	17.5	6.1	23.6	3.9	4.0	7.9	14.2	3.1	17.3
9	...	8.4	8.4	...	12.5	12.5	...	6.75	6.75	...	22.6	22.6
10	1.3	10.8	12.1	1.6	14.2	15.8	1.1	4.95	6.05	1.3	8.6	9.9
11	8.2	1.0	9.2	4.5	10.1	14.6	3.1	1.0	4.1	1.0	16.0	17.0
12	5.9	3.7	9.6	4.8	4.3	9.1	0.32	0.98	1.3	1.8	9.0	10.8
13	7.5	0.9	8.4	3.0	2.7	5.7	0.6	3.45	4.05	1.5	11.0	12.5
14	7.1	1.1	8.2	1.3	5.7	7.0	...	3.55	3.55	...	13.5	13.5
15	1.2	11.0	12.2	...	5.5	5.5	...	1.6	1.6	...	12.5	12.5
16	...	7.3	7.3	...	4.1	4.1	...	1.35	1.35	...	16.8	16.8
17	6.0	...	6.0	0.5	4.7	5.2	...	0.13	0.13	...	9.3	9.3
18	12.2	...	12.2	15.0	...	15.0	2.7	2.8	4.15	1.0	8.4	9.4
19	15.9	...	15.9	20.1	...	20.1	3.25	0.55	3.8	3.1	13.9	17.0
20	...	9.3	9.3	...	4.8	4.8	...	2.55	2.55	...	9.5	9.5
21	17.7	...	17.7	4.0	3.1	7.1	...	0.22	0.22	...	12.7	12.7
22	14.5	...	14.5	5.2	5.1	10.3	0.27	2.43	2.7	1.1	12.8	13.9
23	13.4	...	13.4	17.9	...	17.9	8.15	...	8.15	7.0	7.4	14.4
24	9.2	...	9.2	3.6	4.0	7.6	...	1.35	1.35	...	4.9	4.9
Country (local).	...	5.9	5.9	...	1.9	1.9	...	0.22	0.22	...	8.4	8.4

H. teres was not found in a virulent form up till 1930 but during that year it seems to have been introduced in the Botanical Section, Pusa, through some imported varieties, probably from England or from Nepal. Since then it has established itself and has proved very harmful to certain varieties as will be seen from the above table. During 1930-31 the attack of *H. teres* was bad on types 6, 7 and 21 and some others. Types 7, 12, 17 and 21 were also grown in another field but away from the source of infection, and there was no trace of *H. teres* in them. This fact supports the view that the organism has been introduced.

From Table III it will further be noticed that several types such as types 3, 4, 5, 11, 14, 17, 21 and 24 which got infected during 1930-31 with *H. teres* were either free from or were only lightly attacked by this species but the disease persisted in types 6, 7, 8 and a few others.

The data in Table III show that under the atmospheric conditions at Pusa none of the twenty-four types evolved by selection at this place are completely immune to the disease. The different types however show various degrees of resistance.

The total combined attack on various types varied from year to year. Table IV gives indications of the range of the percentage of leaf area destroyed by *H. teres* and *H. sativum* during the last four years.

TABLE IV

The range of percentage of leaf area destroyed by species of Helminthosporium *on 24 Pusa types of barley*

Organism	PERCENTAGE OF LEAF AREA DESTROYED			
	1930-31	1931-32	1932-33	1933-34
H. teres	0 to 27.9	0 to 25.4	0 to 20.45	0 to 20.7
H. sativum	0 to 11.0	0 to 14.2	0 to 6.75	1.1 to 22.6
Combined effect	2.4 to 27.9	1.2 to 25.4	0.13 to 20.45	4.6 to 23.9

From the above table it will be noticed that the intensity of the disease caused by *H. sativum* was less during 1932-33, but great in 1933-34 when it appeared in several varieties of barley and was responsible for the shrivelling and discoloration of the kernels. Spots extended even on to the awns, and in several cases the seed formed was totally destroyed.

The comparative virulence of *H. sativum* during the last four years on some of the barley types can be judged by an examination of figures for types 3, 4, 5, 9, 14, 16, 19 and 21 in Table III. It will be seen that the attack was most severe in the season 1933-34. The percentage of leaf area destroyed in these types varied in the above-mentioned types from 0 to 1·2 in 1930-31 to 11·2-22·6 in 1933-34,

VIII. Treatment of seed by fungicides to control foot-rot and root-rot and secondary infection

(*i*) *Experiments carried out during 1930-31*

With a view to study the effect of various treatments in checking the disease caused by *H. sativum* and testing the resisting power of certain types of Pusa barleys, four types which showed different degrees of resistance during the previous year were selected for the experiment in 1930-31. The attack of *H. sativum* on these four types during 1929-30 was the same as in Table V.

Table V

H. sativum—*Comparative infection of leaf area during 1929-30*

Date	3-12-29	22-12-29	6-1-30	21-1-30	5-2-30	20-2-30	5-3-30
Type							
7 . . .	S	S	S	F	F	F	B
12 . . .	S	S	S	F	F	F	F
17 . . .	S	S	S	S	S	S	S
21 . . .	VS	VS	VS	VS	S	S	S

Where VS = Leaf infection very slight.
S = Leaf infection slight.
F = Leaf infection fair.
B = Leaf infection bad.

These four types of barley were sown in 96 plots and each treatment for each variety had six replications. Each plot had sixty plants spaced one foot apart.

The following seed treatments were given before sowing :—

A—Control seed sown without any treatment.

B—Seed soaked in water for one hour before sowing.

C—Seed infected heavily with spores of *H. sativum* suspended in water.

D—Heavily infected seed disinfected with a 0·01 per cent solution of mercuric chloride for ten minutes.

Separate germination tests showed that the seed of all the four types under different treatments was almost cent per cent viable.

Death of seedlings due to H. sativum.—A careful weekly record was maintained of the number of seeds which germinated or which died of foot or root-rot caused by *H. sativum*. In some cases a number of seedlings were found to be damaged by white ants. There was no trace of *H. teres* in any of the types. In Table VI is shown the statistical analysis of the data, using Fisher's [1932] method.

TABLE VI

Analysis of variance of percentage death of seedlings due to H. sativum *and effect of seed treatment*

Due to	Degrees of freedom	Sum of squares	Mean squares	x—test	
				Observed value	Expected at $P=0.01$
Blocks	5	2482.0525	496.4105	13.9846	3.017
Varieties	3	10173.2477	3391.0826	95.5321	3.782
Treatments	3	1134.8895	378.2965	10.6572	3.782
Interaction					
Varieties × treatments . .	9	1222.5161	135.8351	3.8265	2.802
Residual error	75	2662.2592	35.4968	...	...
Total .	95	17674.9650	186.0523	...	...

The differences of all possible mean values between the varieties and those between the different treatments are tabulated below. In all tables of mean differences used throughout the text the positive differences which are statistically significant at the one per cent level are given in thick type, whereas those that are significant at the five per cent level are printed in italics.

TABLE VII

Mean differences in the death of seedlings between varieties and between treatments

BETWEEN VARIETIES						BETWEEN TREATMENTS					
Varieties	T. 7	T. 12	T. 17	T. 21	Mean value	Treatment	A	B	C	D	Mean value
T. 7 .	...	—0·14	**24·06**	1·08	17·64	A	...	—0·29	2·74	—6·64	24·94
T. 12 .	0·14	...	**24·20**	1·22	17·50	B	0·29	...	3·03	—6·35	24·65
T. 17 .	—24·06	—24·20	...	—22·98	41·70	C	—2·74	—3·03	...	—9·38	27·68
T. 21 .	—1·08	—1·22	**22·98**	...	18·72	D	**6·64**	**6·35**	**9·38**	...	18·30

Critical difference at one per cent level—4·430.

It will be noted from the above table that during 1930-31 type 17 had about 41·70 per cent deaths in the seedling stage, whereas types 7, 12 and 21 had 17·64, 17·50 and 18·72 per cent respectively. Type 17 showed significantly higher percentage of deaths than all other types while the differences between the others were not significant.

Treatments A, B and C showed average death percentages of 24·94, 24·65 and 27·68 respectively which were not statistically different from each other but treatment D had only 18·30 per cent deaths which is significantly lower. This indicates that by disinfecting the seed before sowing, the destruction of the seedlings due to the disease was minimised. Thus type 17 was most susceptible to the attack of *H. sativum*, and treatment with mercuric chloride helped to reduce the disease during the seedling stage.

Destruction of leaf area by H. sativum.—When the crop was fully grown, the percentage of leaf area destroyed was estimated according to the method already mentioned. Table VIII gives the mean differences in the percentage of leaf area destroyed in the varieties and in the treatments.

TABLE VIII

Mean differences in the percentage of leaf area destroyed between varieties and between treatments

Between varieties						Between treatments					
Varieties	T. 7	T. 12	T. 17	T. 21	Mean value	Treatments	A	B	C	D	Mean value
T 7 .	...	2·44	—4·66	—4·54	6·03	A	...	—1·00	—0·19	—0·73	4·82
T. 12 .	—2·44	...	—7·10	—6·98	8·47	B	1·00	...	0·81	0·27	3·82
T. 17 .	4·66	7·10	...	0·12	1·37	C	0·19	—0·81	...	—0·54	4·63
T. 21 .	4·54	6·98	—0·12	...	1·49	D	0·73	—0·27	0·54	...	4·09

Critical difference is 0.4714 at the one per cent level and 0.3485 at the 5 per cent level.

It will be observed that types 7 and 12 were much more susceptible than types 17 and 21 which is in agreement with the results given in Table VI. Furthermore, type 12 is significantly more susceptible than type 7. The differences between types 21 and 17 on the other hand are not statistically significant.

Considering the percentage leaf destruction obtained in the four treatments, treatments A and C showed significantly higher damage than treatments B and D, but there was no statistical difference between treatments A (control) and C (infected). It is presumed that the incidence of secondary infection from other barleys, wheats or grasses had outweighed any differences due to initial infection.

It may be noticed that during 1930-31 the percentage destruction of leaf area in the four types under study has been inversely proportional to the deaths

in the seedling stage caused by foot or root-rot. This may be seen from the following figures taken from Tables VII and VIII :—

Type	Percentage destruction of leaf area	Percentage death of seedlings by foot-rot and root-rot
17	1·37	41·70
21	1·49	18·72
7	6·03	17·64
12	8·47	17·50

To sum up the results of 1930-31, the experiment indicates that, except in type 17, no increase in infection over the control is obtained by deliberate infection of the seed with spores prior to sowing. This is presumably due to either the soil or the seed already being infected heavily. Infection followed by disinfection reduces the percentage of death from foot-rot and root-rot to well below those of the control in the case of type 17 and to a lesser extent in type 21, while type 12 and type 7 are unaffected. Further, it appears that the more susceptible the variety to foot-rot and root-rot, the smaller is the percentage of destruction of leaf area in the surviving plants.

(ii) Experiments carried out during 1931-32.

During 1931-32 four more types were added to the experiment made in the previous year for the determination of their susceptibility to *H. sativum*. In treatment D of this experiment formalin was used for disinfection instead of mercuric chloride. Infected seeds as in treatment C were disinfected with formalin, 1 : 320, or a solution made by adding one pint of formalin (guaranteed 40 per cent solution of formaldelyde) to 40 gallons of water. The seeds were soaked for twenty minutes in this solution and then were covered up for two hours for the formalin vapour to act. An additional treatment E was introduced in which untreated seed as in treatment A was disinfected with formalin in the manner explained above.

The eight types of barley were sown in 200 plots, each plot having 160 seeds. Each treatment was given five replications. Seedlings which died were removed and examined at regular intervals and recorded.

Death of seedlings.—The percentage of death in seedlings due to foot-rot or root-rot was uniformly high during this year and the results of this experiment are presented in Table IX.

TABLE IX

Mean differences in the death of seedlings between varieties and between treatments

Varieties	BETWEEN VARIETIES								
	T. 1	T. 2	T. 5	T. 7	T. 12	T. 17	T. 20	T. 21	Mean value
T. 1	...	—0·42	2·99	2·84	—3·17	1·02	*4·91*	—4·57	46·66
T. 2 .	0·42	...	3·41	3·26	—2·75	1·44	*5·33*	—4·15	46·24
T. 5 .	—2·99	—3·41	...	—0·15	—6·16	—1·97	1·92	—7·56	49·65
T. 7 .	—2·84	—3·26	0·15	...	—6·01	—1·82	1·93	—7·41	49·50
T. 12 .	3·17	2·75	6·16	6·01	...	4·19	8·08	—1·40	43·49
T. 17 .	—1·02	—1·44	1·97	1·82	—4·19	...	3·89	—4·59	47·68
T. 20 .	—4·91	—5·33	—1·92	—1·93	—8·08	—3·89	...	—9·48	51·57
T. 21 .	*4·57*	*4·15*	7·56	7·41	1·40	*4·59*	9·48	...	42.09

Critical difference at one per cent level = 5·7132
Critical difference at 5 per cent level = 4·3472

Treatments	BETWEEN TREATMENTS'					
	A	B	C	D	E	Mean value
A	...	—2·88	*3·50*	—8·54	—12·43	51·13
B	2·88	...	6·38	—5·66	—9·55	48·25
C	—3·50	—6·38	...	—12·04	—15·93	54·63
D	8·54	5·66	12·04	...	—3·89	42·59
E	12·43	9·55	15·93	*3·89*	...	38·70

Critical difference at one per cent level = 4·518
Critical difference at 5 per cent level = 3·437

The percentage of deaths in the seedling stage due to this disease was invariably very high this year in all the eight types of barley under investigation and ranged from 42·09 per cent in type 21 to 51·57 per cent in type 20. Statistical significance between any two mean values was, therefore, observed only in a few cases and any difference which was higher than the critical difference of 4·3472 per cent was taken as significant.

Although treatments A and B were not significantly different from each other in effect, they showed a higher percentage of deaths in their seedling stages than the disinfected series in treatments D and E. Treatment C had statistically a higher death rate than all other treatments, whereas treatments D and E were the least affected.

Destruction of leaf area.—The average percentage difference of leaf area rendered ineffective by the disease between the different types and the various treatments is shown in Table X.

TABLE X

Mean difference in the percentage of leaf area destroyed between varieties and treatments

Varieties	BETWEEN VARIETIES									BETWEEN TREATMENTS						
	T. 1	T. 2	T. 5	T. 7	7. 12	T. 17	T. 20	T. 21	Mean value	Treat-ments	A	B	C	D	E	Mean value
T. 1	...	1·76	5·00	14·22	6·74	4·66	6·34	4·61	0·14	A	...	0·02	0·60	*1·07*	0·74	5·07
T. 2	—1·76	...	3·24	12·46	4·98	2·90	4·58	2·85	1·90	B	—0 02	...	0·58	*1·05*	0·72	5·09
T. 5	—5·00	—3·24	...	9·22	1·74	—0·34	1·34	—0·39	5·14	C	—0·60	—0·58	...	0·47	0·14	5·67
T. 7	—14·22	—12·46	—9·22	...	—7·48	—9·56	—7·88	—9·61	14·36	D	—1·07	—1·05	—0·47	...	—0·33	6·14
T. 12	—6·74	—4·98	—1·74	7·48	...	2·08	0·40	—2·13	6·88	E	—0·74	—0·72	—0·14	0·33	...	5·18
T. 17	—4·66	—2·90	0·34	9·56	—2·08	..	1·68	—0·05	4·80							
T. 20	—6·34	—4·58	—1·34	7·88	—0·40	—1·68	...	—1·73	6·48							
T. 21	—4·61	—2·85	0·39	9·61	—2·13	0·05	1·73	...	4·75							

Critical difference at one per cent level = 1·0896.
Critical difference at 5 per cent level = 0·8291.

Critical difference at one per cent level = 1·3242.
Critical difference at 5 per cent level = 1·0058.

It will be evident that barley type 7 suffered the most from secondary infection and showed an average destruction of 14·36 per cent of its leaf area. This value was statistically higher than that shown by any other type. The other six-rowed barleys, *viz.*, types 12, 20, 17 and 21, respectively, had 6·88, 6·48, 4·80 and 4·75 per cent of their leaf areas damaged by the fungus. Of the two-rowed barleys, type 5 was the worst affected having 5·14 per cent destruction, whereas types 2 and 1 had losses of only 1·90 and 0·14 per cent respectively. It may be added that type 1 is the earliest maturing Pusa barley and as such escapes secondary infection to a very great extent. These results again prove that the incidence of secondary infection, causing leaf destruction by *H. sativum*, brings about inappreciable differences between the control, the infected and the disinfected series. In other words, disinfecting the seed does not help in checking secondary infection in mature plants to any appreciable extent, though it helps in reducing the disease in the primary phase.

In the four types of barley that were studied in the previous year it was found that the percentage destruction of leaf was inversely proportional to the deaths caused by *H. sativum* in the seedling stage. This year, however, this fact was not corroborated as will be apparent from the following figures selected from Tables IX and X.

Type	Percentage destruction of leaf area	Percentage deaths of seedlings by foot-rot and root-rot
1	0.14	46.66
2	1.90	46.24
21	4.75	42.09
17	4.80	47.68
5	5.14	49.65
20	6·48	51.57
12	6·88	43.49
7	14·36	49.65

Effect of different treatments on the incidence of H. sativum *on barley types 7 and 21 in 1931-32.*—In addition to treatments A to E the results of which have already been enumerated above, four other disinfectants were tried this year in another set of experiments with types 7 and 21 only. The eight treatments used were :—

Northern portion of the field—

A. Control—seed sown dry in the usual way.

B. Seed soaked in water for one hour before sowing.

C. Seed infected heavily with spore suspension in water and sown.

D. Infected seed as in C disinfected with formalin (Formalin 1 : 320, or a solution made by adding a pint of formalin to 40 gallons of water. Seeds kept soaked for 20 minutes and then under formalin vapour for two hours).

E. Seed disinfected with formalin as above.

Southern portion of the field—

F. Control—seed sown dry in the usual way as in A.

G. Seed as in A disinfected with formalin as in D.

H. Seed as in A disinfected with uspulun (0·25 per cent solution for one hour).

I. Seed as in A disinfected with mercuric chloride (1 : 1000) for ten minutes and then washed in running water before sowing.

J. Seed as in A disinfected by dusting with sulphur at the rate of four ounces of fine sulphur (No. 120) per 60 lb. of seed.

Difference between the two types of barley

Type	Average percentage of leaf area destroyed	Average percentage of death of seedlings
7	13·24	41·96
21	4·78	36·16
Critical difference at one per cent level	0·87	2·42

Type 7 in both cases shows statistically higher casualties.

Difference between the various treatments

Treatment	Average percentage of leaf destroyed	Average percentage of death of seedlings
A	9·04	49·68
B	8·80	47·44
C	10·17	55·08
D	10·52	42·59
E	9·25	34·21
F	7·77	41·76
G	7·02	36·06
H	8·37	24·19
I	9·17	27·26
J	9·99	31·31
Critical difference at 5 per cent level	1·48	9·21
Critical difference at one per cent level	1·95	12·12

It will be apparent from the above table that as far as the destruction of leaf area is concerned the infected series C shows statistically higher destruction than F, G and H only, and that it is not significantly higher than the other treatments.

As regards the average percentage of seedling deaths, C (infected) is certainly the worst and treatments H, I, J the best. There is no statistical difference, however, between treatment C and either A or B. It may be concluded that the different disinfection treatments used in this experiment minimise the effect of the disease in barley.

In another experiment during 1931-32 seeds of 24 types of Pusa barleys were disinfected with formalin and uspulun to control the disease. The treatment was conducted as follows : (*a*) Control, no treatment ; (*b*) seeds disinfected with formalin as described before ; (*c*) seeds disinfected with uspulun (universal), 0·25 per cent solution for one hour. The extent of damage to the leaves was as shown below :—

TABLE XI

The extent of damage to leaf area in different types of barley in 1931-32

Damage	Control (No treatment)	Formalin treatment	Uspulun treatment
Very slight . . .	*Nil*	Two types—2, 3 . .	Six types—1, 2, 3, 4, 5, 17
Slight	Two types—2, 3 . .	Five types—1, 4, 5, 17, 21	Twelve types—11, 12, 13, 15, 16, 18, 19, 20, 21, 22, 23, 24.
Fair	Thirteen types—1, 4, 5, 12, 13, 14, 15, 16, 17, 20, 21, 22, 24.	Fifteen types—8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 22, 23, 24.	Five types—7, 8, 9, 10, 14
Bad	Nine types—6, 7, 8, 9, 10, 11, 18, 19, 23.	Two types—6, 7 . .	One type—6.

The above table confirms the previous results that uspulun treatment controls the disease to a certain extent and is more effective than formalin treatment.

Effect of various treatments on the yield of barley types

In 1931-32 the final yields of all the plots under the eight types of barley were taken and statistically analysed in order to see whether artificial infection reduced the yield and also whether disinfection with formalin increased it significantly. Table XII presents the yields in decagrammes obtained per plot.

TABLE XII

Yields in decagrammes of eight types of barley under five different seed treatments

Treatment	Replication	VARIETIES								Treatment totals
		T. 1	T. 2	T. 5	T. 7	T. 12	T. 17	T. 20	T. 21	
A . .	1	9	4	4	27	31	13	31	58	177
	2	45	4	4	94	103	45	99	145	539
	3	45	4	4	14	126	49	72	144	458
	4	63	18	13	76	117	81	112	148	628
	5	31	13	22	108	139	90	44	184	631
										2,433
B . .	1	31	13	4	58	81	9	72	67	335
	2	18	4	4	54	81	36	81	85	363
	3	45	9	13	112	139	58	108	166	650
	4	54	9	4	117	130	58	121	166	659
	5	58	4	22	54	72	22	63	144	439
										2,446
C . .	1	18	9	4	58	90	22	72	90	363
	2	58	9	9	67	144	36	45	63	431
	3	63	9	9	121	139	45	117	126	629
	4	67	13	9	94	108	67	67	144	569
	5	22	9	18	49	81	45	117	94	435
										2,427
D . .	1	27	13	4	58	81	54	76	103	416
	2	72	9	9	112	117	36	67	162	584
	3	90	27	4	121	157	81	112	157	749
	4	54	9	9	108	135	81	67	139	602
	5	31	9	4	72	117	45	117	81	476
										2,827
E . .	1	36	9	4	108	112	45	81	126	521
	2	45	13	9	117	135	58	108	193	678
	3	9	9	22	144	148	72	112	121	637
	4	31	22	13	108	189	90	108	139	700
	5	36	22	22	108	135	76	121	166	686
										3,222
Varietal totals .	..	1,058	273	243	2,159	2,907	1,314	2,190	3,211	13,355

The analysis of variance for these data is shown in the following table.

TABLE XIII

Analysis of variance

Due to	Degrees of freedom	Sum of squares	Mean square	Value of $\frac{\text{variance 1}}{\text{variance 2}}$	
				Observed	Expected at $P = \cdot 01$
Varieties	7	356143·435	50877·6335	116·394	2·802
Treatments	4	12368·550	3092·1375	7·074	3·320
Replications	4	29628·650	7407·1625	16·956	3·320
Interactions.					
Varieties × treatments	28	15610·690	557·5246	1·275	1·791
Treatments × replications	28	16897·050	603·4661	1·385	1·791
Residual error	128	55950·500	437·1132	...	...
Total	199	486598·875	2441·1953	...	...

It will be noticed that the mean square contributed by the varieties is the greatest and is followed by that for replications and treatments.

The differences in mean yields between the five treatments studied are shown below :—

TABLE XIV

Mean differences between treatments

Treatments	A	B	C	D	E	Mean yield
A	..	0·325	—0·150	*9·850*	**19·725**	60·825
B	—0·325	..	—0·475	*9·525*	**19·400**	61·150
C	0·150	0·475	..	*10·000*	**19·875**	60·675
D	—9·850	—9·525	—10·000	..	*9·875*	70·675
E	—19·725	—19·400	—19·875	—9·875	..	80·550

Critical difference at one per cent level is 12·042 and at 5 per cent level is 9·163.

It is seen that the combined mean yields of all the eight types of barley under treatments A, B and C are 60·825, 61·150 and 60·675 decagrammes respectively and the difference between any two of these values is not significant. The mean yields of the disinfected series D and E are 70·675 and 80·550 decagrammes

respectively. These average values are statistically greater than those in the other treatments. Treatment E especially yielded the highest. Disinfection with formalin therefore has produced significantly higher yields in barley because of the fact that it controls the disease to some extent. This will be evident also from a comparison of the yields of each type under the different treatments.

TABLE XV

Mean yields in decagrammes of different types of barley under various seed treatments

Treatment	Barley types							
	1	2	5	7	12	17	20	21
A	193	43	47	319	516	278	358	679
B	206	39	47	395	503	183	445	628
C	228	49	49	389	562	215	418	517
D	274	67	30	417	607	297	439	642
E	157	75	70	585	719	341	530	745

Treatments D and E (disinfected series) show higher yields than the other treatments in most of the barley types under investigation.

(iii) Experiments carried out during 1932-33 and 1933-34

In these two years all the twenty-four types of barley evolved at Pusa were tried. During 1932-33 the seed was treated before sowing as follows :—

A. Control—no treatment.

B. Seed soaked in water for one hour.

C. Seed infected heavily with spore suspension in water.

D. Infected seed as in treatment C disinfected with uspulun (universal) for one hour.

E. Control seed as in treatment A disinfected with uspulun (universal).

During 1933-34 an additional sixth treatment, F, was given in which infected seed as in C was disinfected with ceresan at the rate of two ounces per bushel of seed. Each of the twenty-four types had six replications for each of the treatments during 1932-33 and seven during 1933-34.

The virulence of the disease as expressed both by deaths in the seedling stage as well as by the destruction of leaf area was definitely greater in 1933-34 than in 1932-33.

The average percentage loss due to seedling mortality and to destruction in leaf area in the twenty-four types of Pusa barley, irrespective of seed treatments, is shown in Table XVI. If the differences between the average values of any two types in this table are greater than the expected critical difference shown at the bottom of each column, the differences may be gauged to be statistically significant.

TABLE XVI

Average percentage loss in different types of Pusa barleys due to the attack of H. sativum

Type	Average percentage of seedling mortality		Average percentage of leaf area destroyed	
	1932-33	1933-34	1932-33	1933-34
1	17·64	12·46	0·01	3·32
2	17·90	14·86	0·46	8·00
3	21·93	14·60	1·59	9·78
4	23·59	16·87	2·27	7·14
5	28·39	21·06	2·74	8·50
6	12·61	13·81	13·37	14·22
7	11·10	11·45	8·26	17·46
8	12·15	11·92	5·81	15·37
9	10·47	11·47	1·31	18·36
10	11·81	11·96	1·00	14·47
11	14·05	12·70	3·39	11·33
12	8·62	14·90	0·46	11·14
13	17·86	20·23	0·63	17·41
14	22·82	27·24	0·71	13·68
15	20·67	17·40	1·92	18·29
16	8·62	18·47	0·35	10·99
17	9·49	15·05	0·01	7·64
18	13·39	20·95	0·75	5·23
19	10·93	10·90	0·80	12·11
20	13·40	11·33	0·37	10·93
21	16·44	10·12	0·22	8·45
22	10·75	16·38	0·39	11·38
23	15·45	14·44	0·89	14·45
24	19·78	22·05	0·23	4·18
General mean	15·386	15·526	1·998	11·823
Standard error of difference	1·908	1·380	0·224	0·306
Critical difference at one per cent level	3·740	3·555	0·576	0·775

The results are obvious and hence tables of differences are hardly necessary. It may be concluded that the various types of Pusa barley respond differentially to this disease and that the seasonal conditions play an important role in the incidence of the disease on the different varieties tried. Types 1 to 5 belong to the two-rowed species of barley and tend to show less destruction of leaf area than the other types which are six-rowed barleys.

Tables XVII and XVIII show the mean differences observed under various treatments in the two seasons, 1932-33 and 1933-34 in respect of percentage of seedlings which died and for the destruction of leaf area.

TABLE XVII

Average differences in percentage of loss in different seed treatments due to the attack of H. sativum*—1932-33*

Treatments	Death in seedling stage						Destruction of leaf area						
	A	B	C	D	E	Mean value	Treatments	A	B	C	D	E	Mean value
A . . .	...	0·78	—0·14	—6·34	—5·07	17·54	A . .	...	—0·471	**0·771**	—0·013	—0·913	1·935
B . . .	—0·78	...	—0·92	—7·12	—5·85	18·32	B . .	—0·471	...	**0·300**	—0·484	—1·384	2·406
C . . .	0·14	0·92	...	—6·20	—4·93	17·40	C . .	—0·771	—0·300	...	—0·784	—1·684	2·706
D . . .	**6·34**	**7·12**	**6·20**	...	1·27	11·20	D .	0·013	**0·484**	**0·784**	...	—0·900	1·922
E . . .	**5·07**	**5·85**	**4·93**	—1·27	...	12·47	E . .	**0·913**	**1·384**	**1·684**	**0·900**	...	1·022

Critical difference at the one per cent level — 1·944

Critical difference at the one per cent level — 0·263

TABLE XVIII

Average differences in percentage of loss in different seed treatments due to the attack of H. sativum*—1933-34*

Treatments	Death in seedling stage							Destruction of leaf area						
	A	B	C	D	E	F	Mean value	Treatments	A	B	C	D	E	F	Mean value
A . .	...	—0·31	—3·36	—10·19	—11·93	—15·51	22·41	A . .	..	**0·988**	—1·423	—0·709	—0·515	—4·001	12·767
B . .	0·31	...	—3·05	—9·88	—11·62	—15·20	22·10	B . .	—0·988	...	—2·411	—1·697	—1·503	—4·989	13·755
C . .	**3·36**	**3·05**	...	—6·83	—8·57	—12·15	19·05	C . .	**1·423**	**2·411**	...	**0·714**	**0·908**	—2·578	11·344
D . .	**10·19**	**9·88**	**6·83**	...	—1·74	—5·32	12·22	D . .	**0·709**	**1·697**	—0·714	...	0·194	—3·292	12·058
E . .	**11·93**	**11·62**	**8·57**	**1·74**	...	—3·58	10·48	E . .	**0·515**	**1·503**	—0·908	—0·194	...	—3·486	12·252
F . .	**15·51**	**15·20**	**12·15**	**5·32**	**3·58**	...	6·90	F . .	**4·001**	**4·989**	**2·578**	**3·292**	**3·486**	...	1·066

Critical difference at the one per cent level—1·771

Critical difference at the one per cent level—0·387

It will be noted from the two tables given above that artificial infection (treatment C) has hardly had any appreciable difference on the death of seedlings caused by this fungus, and as a matter of fact the percentage of deaths appeared to be somewhat lower than in the treatments A and B which were taken as controls. This reduction may possibly be due to the fact that at the time of artificial infection, which was done in a suspension of spores in water, the lighter and weak seeds which usually float on the surface of the water and which perhaps were more susceptible to the disease were inadvertently thrown away, and hence there was an elimination of some susceptible material and only healthy and plump seeds remained to be sown under treatment C.

The disinfected series D, E and F have definitely shown less damage both in the seedling stage as well as on the leaf area. Ceresan (treatment F) especially appears to have controlled the disease better than uspulun (treatments D and E). In 1932-33 the highest damage due to secondary infection was shown by treatment C (artificially infected) where the mean value worked out to be 2·706 per cent. The second and third places were taken by treatments B (seed soaked in water before sowing) and A (seed sown dry), respectively, with mean values of 2·406 per cent and 1·935 per cent respectively. In treatment D (artificially infected and then disinfected with uspulun) the mean leaf area infected by *H. sativum* was 1·922 per cent, a value which was not statistically different from the values obtained for treatments A, B and C. Treatment E, however, in which ordinary seed similar to that in treatment A was disinfected with the same strength of uspulun, showed only 1·022 per cent damage and this value was statistically less than that shown by the other treatments. This indicates that uspulun was effective in controlling the disease to an appreciable extent in treatment E but not so in treatment D because of the additional artificial infection given immediately before disinfection. Secondary infection appears to have reduced the variability due to the different treatments except F in 1933-34.

Considering the average percentage loss in treatment A very low correlations were found to exist between :—

1. Seedling deaths in 1932-33 and those in 1933-34 ($r = 0{\cdot}0980 \pm 0{\cdot}1364$),
2. Leaf area destruction in 1932-33 and that in 1933-34 ($r = 0{\cdot}0944 \pm 0{\cdot}1365$),
3. Seedling mortality and leaf area destruction in 1932-33 ($r = -0{\cdot}1018 \pm 0{\cdot}1362$),
4. Seedling mortality and leaf area destruction in 1933-34 ($r = -0{\cdot}0374 \pm 0{\cdot}1375$).

IX. Seasonal effect

That season exerts a great deal of influence on the incidence of *H. sativum* and consequently on the damage caused by this fungus to the barley plant is a fact which cannot be contested and has already been demonstrated by the results

enumerated in the preceding pages. A statistical proof of this can be obtained by analysing in detail the behaviour of types 7, 12, 17 and 21 during the four years 1930-31 to 1933-34.

In Table XIX is presented the complete analysis of variance of the percentage of deaths in the seedling stage and of the percentage leaf area destroyed by *H. sativum* respectively. It will be noted that for both these characters the largest figure for variance or mean square has been contributed by the variance due to seasons, and specially in the case of seedling mortality this figure is very pronounced and very much higher than that contributed by any other variables proving thereby that season exerts a great deal of influence in the variability of the disease affecting different types of barley.

TABLE XIX

Analysis of variance of percentage of death seedlings and leaf area destruction respectively due to Helminthosporium *from 1930-31 to 1933-34*

Due to	Degrees of freedom	Death of seedlings			Leaf area destruction			Expected ratio of $(S_1)^2/(S_2)^2$ at one per cent level
		Sum of squares	Mean squares $(S_1)^2$	Observed ratio— $(S_1)^2/(S_2)^2$	Sum of squares	Mean squares $(S_1)^2$	Observed ratio— $(S_1)^2/(S_2)^2$	
Blocks	4	496·8615	124·2154	7·142	27·60	6·90	11·696	3·320
Varieties	3	3029·3550	1009·7850	58·063	3494·22	1164·74	1974.234	3·782
Seasons	3	63463·9420	21154·6473	1216·392	4261·82	1420·61	2407·934	3·782
Treatments	3	3949·6472	1316·5491	75·702	31·36	10·45	17·713	3·782
Interaction :—								
Varieties × blocks	12	474·6170	39·5514	2·274	77·20	6·433	10·904	2·182
Treatments × seasons	9	3033·5833	337·0648	19·381	108·22	12·024	20·381	2·511
Seasons × blocks	12	2783·0570	231·9214	13·335	137·07	11·423	19·362	2·182
Treatments × blocks	12	905·6262	75·4688	4·339	38·44	3·203	5·429	2·182
Treatments × varieties	9	1037·8183	115·3131	6·631	152·43	16·951	28·708	2·511
Varieties × seasons	9	8648·7195	960·9688	55·256	886·81	98·534	167·015	2·511
Treatments × seasons × blocks	36	2439·5783	67·7661	3·897	182·90	5·085	8·619	1·000
Treatments × seasons × varieties	27	5025·1702	186·1174	10·702	438·52	16·241	27·528	1·791
Seasons × varieties × blocks	36	1331·5993	36·9887	2·127	427·16	11·866	20·113	1·000
Treatments × varieties × bolcks	36	2494·1535	69·2820	3·984	88·76	2·466	4·180	1·000
Residual error	108	1877·6672	$17.3858(S_2)^2$	..	63·37	$.59(S_2)^2$	..	..
Total	319	100991.3955	316.5874		10415.88	32.6526		

The average values of the number of seedlings that died and the leaf area destroyed in the whole experiment, irrespective of varieties and treatments, during the four seasons in which this experiment was conducted, together with all possible differences are shown in Table XX.

TABLE XX

Mean differences between seasons

Death of seedlings						Leaf area destroyed					
Season	1930-31	1931-32	1932-33	1933-34	Mean value	Season	1930-31	1931-32	1932-33	1933-34	Mean value
1930-31	...	**23·93**	—12·02	—8·86	24·51	1930-31	...	**3·25**	—1·83	**7·78**	4·43
1931-32	—23·93	...	—35·95	—32·79	48·44	1931-32	—3·25	...	—5·08	**4·53**	7·68
1932-33	**12·02**	**35·95**	...	**3·16**	12·49	1932-33	**1·83**	**5·08**	...	9·61	2·60
1933-34	**8·86**	**32·79**	—3·16	...	15·65	1933-34	—7·78	—4·53	—9·61	...	12·21

Critical difference at one per cent = 1·698 Critical difference at one per cent = 0·310

It is evident that in the four barley types under study *H. sativum* brought about 12·49 per cent deaths in the seedling stage in 1932-33, 15·65 per cent in 1933-34, 24·51 per cent in 1930-31 and 48·44 per cent in 1931-32. The destruction of leaf area was least in 1932-33 working out to be only 2·60 per cent and maximum in 1933-34 when it was 12·21 per cent. In 1930-31 it was 4·43 per cent, while in 1931-32 it was 7·68 per cent. The positive differences which are statistically significant are in thick type in the above table.

The behaviour of these four types of barley during the seasons under review may be gauged from the table which follows :—

TABLE XXI

Mean differences between varieties of barley

Death of seedlings						Leaf area destroyed					
Varieties	T. 7	T. 12	T. 17	T. 21	Mean value	Varieties	T. 7	T. 12	T. 17	T. 21	Mean value
T. 7 .	...	—1·67	**6·21**	—0·61	24·29	T. 7 .	...	—4·24	—8·42	—7·54	11·78
T. 12 .	*1·67*	..	**7·88**	1·06	22·62	T. 12 .	**4·24**	...	—4·18	—3·30	7·54
T. 17 .	—6·21	—7·88	...	—6·82	30·50	T. 17 .	**8·42**	**4·18**	..	**0·88**	3·36
T. 21 .	0·61	—1·06	**6·82**	...	23·68	T. 21 .	**7·54**	**3·30**	—0·88	...	4·24

Critical difference at one per cent = 1·698
Critical difference at 5 per cent = 1·292

Critical difference at one per cent = 0·310
Critical difference at 5 per cent = 0·236

Type 17 seems to show maximum and type 12 the minimum percentage of deaths during the seedling stage, whereas type 7 shows the maximum and type 17 the minimum destruction of leaf area due to the fungus.

Similarly, the differences of mean values due to the various seed treatments are presented in Table XXII below :—

TABLE XXII

Mean differences between different seed treatments

Treatments	Death of seedlings					Treatments	Leaf area destroyed				
	A	B	C	D	Mean value		A	B	C	D	Mean value
A .	...	—1·52	—1·39	—8·97	28·24	A . .	..	0·48	0·73	—0·02	6·43
B .	*1·52*	...	0·13	—7·45	26·72	B . .	—0·48	...	*0·25*	—0·50	6·91
C .	*1 39*	—0·13	...	—7·58	26·85	C . .	—0·73	—0·25	...	0·75	7·16
D .	8·97	7·45	7·58	...	19·27	D . .	0·02	0·50	0·75	...	6·41

Critical difference at one per cent = 1·698
Critical difference at 5 per cent = 1·292

Critical difference at one per cent = 0·310
Critical difference at 5 per cent = 0·236

The results are too obvious to need further comments and it is apparent that seed disinfection reduced death during the seedling stage but had no appreciable effect in reducing the secondary attack.

X. CONCLUSION

H. sativum causes considerable damage to the barley crop in Pusa every year. The amount of infection varies with locality, year and the variety of barley. All Pusa types of barley are more or less susceptible to the disease. It has been found that certain ecological factors, especially temperature, play an important part in varying the severity of the disease. *H. teres* also has recently been introduced at Pusa but is restricted to certain types only, whereas *H. gramineum* is either absent or very rare and occurs only during certain years when a foreign type is grown at the Pusa Farm. This is perhaps due to the fact that the range of temperature at Pusa is not suitable for the growth of *H. gramineum*. According to Johnson [1925] low soil temperature (10° to 12°C.) favours infection of barley and very little stripe disease occurs at soil temperatures higher than 20° C. This low temperature does not occur at Pusa at the time of sowing or harvesting and this may account for the absence of the disease. In the United Provinces and also in Nepal, where the soil temperature is lower, this disease makes its appearance. That high temperatures are favourable for the development and the spread of the disease caused by *H. sativum* has also been noticed by Dosdall [1923], McKenny [1923] and Henry [1924].

Predisposition plays an extremely important part in the degree of infection, *e.g.*, type 21, which is less susceptible, was rather severely attacked during 1933-34. This may be due to the conditions unfavourable for the growth of the barley plant but favourable for the growth of the fungus. Thus, when a resistant type is confronted by unfavourable conditions, it also becomes liable to infection.

The reaction of the soil also determines to a great extent the degree of infection, and this perhaps is the reason why infection is more in certain fields and less in others or is greater in one part of the field and milder in another part of the same field.

Weather conditions at harvest time or just before it, exert an influence on the percentage of infection in the ear and grain. Dry weather at the critical period tends to reduce the intensity of contamination while a wet period favours the development of *H. sativum*.

Various fungicides tried during the four years under review helped to check the disease to a certain extent. The superiority of uspulun in controlling the disease was confirmed several times during 1931-32, 1932-33 and 1933-34 and ceresan which was tried during 1933-34 proved to be still more effective. Though these fungicides controlled the disease to a greater or lesser extent during the seedling stage they usually failed to check the attack on the leaf surface owing to secondary infection taking place by means of air-borne spores from different fields of barley, wheat and other wild grasses on which *H. sativum* thrives. Further, *H. sativum* is capable of living saprophytically in the soil and can infect treated seed. It has been noticed that the plots on which barley is grown year after year are the worst affected both during the seedling and the mature stages of the plant.

It is not sufficient only to disinfect barley seed one year and save the grain from the resultant crop for sowing in the succeeding year, as such grain may be heavily contaminated with spores and mycelium of *H. sativum* due to secondary infection. It is essential that seed should be treated every year with an effective disinfectant of the ceresan type, if the disease is to be controlled in the primary phase. As far as the obvious loss is concerned, the primary phase (foot-root and root-rot) is of course the more important than the secondary infection on account of the greater mortality during the seedling stage.

Since the disease, especially the secondary infection, cannot effectively be controlled by seed treatment alone, the necessity for evolving new types resistant to the disease becomes apparent. It has been observed that there is a differential varietal resistance in the Pusa types of barley, that is, some are susceptible while others are resistant to a certain degree. The use of existing resistant varieties and breeding of new types with more suitable agronomic characters is therefore one of the most promising methods of preventing *Helminthosporium* disease. This method is receiving the attention it deserves at Pusa. In addition to breeding resistant varieties, it is advisable to plant clean seed and to have crop rotation in order to reduce the possibility of infection from the soil.

XI. Summary

1. Three species of *Helminthosporium* occur on barley in India, *viz.*, *H. sativum* P. K. & B., *H. teres* Sacc. and *H. gramineum* Rabh.

2. *H. sativum* is common at Pusa and in its neighbourhood. It is responsible for foot-rot and root-rot, head blight and spot formation on all aerial parts. It lowers the percentage of seed germination and reduces the crop yield.

3. *H. teres* also occurs at Pusa but is restricted to such types as have been introduced from outside. It is altogether absent on the types of barley grown locally. *H. gramineum* is very rare at Pusa.

4. The amount of damage done by *H. sativum* to various types of Pusa barley, varies from type to type, from season to season, and from plot to plot. In some years it occurs in a virulent form, *e.g.*, during 1934.

5. The percentage of leaf surface destroyed by *H. sativum* and *H. teres* during 1930-1934 on various types of barley is given.

6. Various fungicides were used to control the disease, *i.e.*, sulphur, formalin, uspulun, mercuric chloride and ceresan. Mercuric compounds were the most successful but none of the disinfectants was found to check the disease entirely. This is due to the fact that the organism lives in the soil and is parasitic on several grasses and also on wheat. Thus, repeated infections may occur during the growing season and seed treatment may not eliminate the disease altogether. Suitable treatment of each year's seed will, however, check the disease during the more important primary phase.

7. Differences exist in the susceptibility of different varieties to the attack of *H. sativum* but the degree of infection is influenced by environmental factors such as temperature, humidity, etc.

8. Since the disease, especially the secondary infection, cannot be effectively controlled by seed treatment alone, the study of varietal resistance is of great importance.

The writers are very grateful to Mr. Azmatullah Khan, Fieldman, Mycological Section, Pusa, for the ungrudging help rendered in making observations of various field experiments during the course of this study.

REFERENCES

Bose, R. D. (1931). *Ind. J. Agric. Sci.* **1**, 58-89.

Christensen, J. J. (1922). *Minn. Agric. Expt. Stn. Tech. Bul.* **11.**

——————— (1926). *Minn. Agric. Expt. Stn. Tech. Bul.* **37.**

Dosdall, L. T. (1923). *Minn. Agric. Expt. Stn. Tech. Bul.* **17.**

Henry, A. W. (1924). *Minn. Agric. Expt. Stn. Tech. Bul.* **22.**

Johnson, T. (1925). *Phytopath.* **15**, 797-804.

McKenny, H. H. (1923). *J. Agric. Res.* **26**, 185-218.

Mitra, M. (1931, 1). *Trans. Brit. Myc. Soc.* **15**, 254-293.

——————— (1931, 2). *Trans. Brit. Myc. Syc.* **16**, 115-127.

——————— (1931, 3). *Report of the Imperial Mycologist—Sci. Report of the Imp. Inst. of Agric. Res., Pusa* (1929-30), pp. 58-71.

Stevens, F. L. (1922). *Ill. Dept. of Reg. & Educ., Div. Nat. Hist. Surv.* **14**, Art. 5, 77-185.

Tehon, L. R. (1927). *Ill. Dept. of Reg. & Educ., Div. Nat. Hist. Surv.* **17**, Art. 1, 1-96.

SEEDLING BLIGHT OF *CINCHONA LEDGERIANA* MOENS CAUSED BY *PHYTOPHTHORA PALMIVORA* BUTL. IN THE DARJEELING DISTRICT

BY

K. F. KHESWALLA, B.A.,

Assistant to the Imperial Mycologist, Imperial Institute of Agricultural Research, Pusa, Bihar

(Received for publication on 25th February 1935)

(With Plate XX)

CONTENTS

I. INTRODUCTION

Cultivation of cinchona was taken up in India on or about 1866 at which time Sir Clements R. Markham brought the seed of *Cinchona officinalis* L. and *C. Calisaya* Weddel from Bolivia and Peru. The first plantation was laid down in the Nilgiri Hills of the Madras Presidency and since then its cultivation has been extensively taken up in the Sikkim plantations of the Darjeeling district and in

certain parts of Burma. The cinchona plant does not grow below an altitude of 2,000 feet. The crop needs copious rainfall, a cool and humid climate and at the same time well-drained land, as water-logging is fatal to its growth. In the Darjeeling district it is grown on steep mountain sides and all attempts to grow these plants in the plains were unsuccessful.

A serious seedling-blight disease was first observed at Mungpoo, in the Darjeeling district in 1928 in seed-beds and, as it was causing a good deal of loss to the plantation, the investigation was taken up.

II. Symptoms

The disease manifests itself for the first time at the collar of an affected seedling where the discoloration of affected tissues becomes evident. The discoloured area gradually extends above till it reaches the cotyledons which then become limp and bend over. The leaves gradually become yellow, curl inwards, and in severe cases are shed, but this is not so very common. The discoloration is followed by rotting and when the decomposition of the bark tissues has started and the inner vascular regions are affected the seedlings hang down from the affected portion and die.

When the weather is moist, copious aerial mycelium can be seen on the affected seedling and microscopic examination reveals the presence of numerous sporangia attached to broad unseptate mycelium indicating that the pathogene is a Phycomycete. The mycelium is broad, with a granular protoplasm, and penetrates the bark, cortex and the wood. It is found both in the cells and between the cells. When the mycelium penetrates the cell wall, it becomes constricted just at the point of perforation. Haustoria are not observed. The cell contents are destroyed and in advanced cases the walls collapse. The sporangia are rather loosely attached to the stalks and at the slightest puff of wind they are blown to other plants that are near, and the disease may spread in this manner also. Several seedlings at Mungpoo were found to die from the top downwards, though they were affected by the same Phycomycete, indicating that the infection can also be aerial, that is, from the sporangia that are blown about from the affected seedlings or by zoospores which may also be splashed by rain on to the leaves and may bring about aerial infection. A close search for oospores on diseased seedlings was made but none were found.

III. Isolation

Pieces of affected seedlings were first surface-sterilised by washing them in 0·1 per cent corrosive sublimate solution and then in several changes of sterile water. They were then incubated in moist chambers. A copious growth of mycelium was found on the incubated tissues in about five days time; the mycelium was profusely sporulating. Bits of this mycelium were transferred to Quaker oat agar tubes and culture of the fungus was thus obtained. Single sporangial cultures were then obtained by using Keitt's [1915] plating method. The fungus isolated was a species of *Phytophthora*.

PLATE XX

A seedling of *Cinchona ledgeriana* infected with *Phytophthora palmivora* Butl. (natural size)

IV. Pathogenicity

Inoculation experiments with *Phytophthora* isolations were carried out at Pusa and at Munsong and Sureil near Mungpoo in the Darjeeling district. For this purpose, seedlings of cinchona that were about one foot high were selected and brought down to Pusa for inoculation purposes. The inoculum was placed either on the base of the stem or on the tender leaves.

After placing the infective material in position, it was covered with wet cotton wool and the cotton was kept constantly moist. The results of the test are recorded in Table I.

Table I

Results of inoculating cinchona seedlings with a species of Phytophthora

No. of plants inoculated	No. of plants taking infection	Position where inoculum was placed
15	9	Leaves
15	15	,,
15	10	,,
7	7	Collar
10	6	,,

In each case five plants were kept as controls. The symptoms on the inoculated plants (Plate XX) were similar to those noticed on naturally infected plants. The controls received the same treatment as the infected plants except that the infective material was not placed on them. All of them remained quite healthy during the course of the experiments and much later. From the infected plants the causal organism was isolated and it agreed with the original fungus. The pathogenicity of *Phytophthora* sp. isolated from the diseased seedlings was thus established. At the time the inoculations were done at Pusa the temperature was in the neighbourhood of 27°C. and the humidity was very high. At Mungsong where the right conditions for getting successful inoculations were available, infection experiments carried out also proved the pathogenicity of the fungus.

V. Morphology

The morphological features of the organism were studied on Quaker oat agar, a few other *Phytophthora* spp. being also included in this study for purposes of comparison, *viz.*, *P. palmivora* Butl., *P. meadii* McRae, *P. colocasiae* Rac., and *P. parasitica* Dast. It was noted that while these species put forth profuse aerial

growth, the cinchona *Phytophthora* was less profuse. Macroscopically it appeared to bear resemblance to *P. palmivora* and to a less extent to *P. meadii* than to the other fungi included in the study. The aerial hyphae appeared to be rather broader than the submerged hyphae, the hyphae were unseptate when young but put forth thin septa at the time of forming the fruit bodies. The mycelium was richly granular and became knotted in some cases. In cultures the sporangia were formed rather sparingly. They were narrowly and in some few cases broadly ovate, pear-shaped, ellipsoidal or sometimes almost spherical. The papillae were very prominent. The sporangium bore at the basal end a distinct pedicel. They were mostly borne terminally but a few were intercalary. For obtaining a large number of sporangia, a bit of the vigorously growing mycelium was transferred to plain, sterile water and incubated overnight at 20°C. Sporangia thus formed were smaller in size than those obtained in culture, which might possibly be due to lack of food material.

Germination of the sporangia may be direct or indirect. In the former case slightly higher temperatures were observed to be necessary but indirect germination by means of zoospores through the papillate end of a sporangium was favoured by low temperatures. Each sporangium may put forth zoospores which swim for a while, become round and then come to rest ; the number of zoospores varied from 13 to 29 according to the size of the sporangia. In the case of direct germination by means of germ tubes, secondary sporangia may be formed which produce the zoospores. Two hundred sporangia were measured from a water culture at 25°C. and the range for length and breadth was 17·5—58·5μ and 9·0—30·0μ, mean being 33·3 × 17·6μ. In old cultures chlamydospores predominate and measurement of 200 of them indicated that they had an average diameter of 37·3 μ. A statistical analysis of the spore measurements is given later. Oospores were found neither in the host tissues nor in artificial cultures.

VI. Influence of environment on spore production

(*a*) *Humidity*

Slant cultures on Quaker oat agar were kept in a desiccator for comparison with similar slants at laboratory humidity. The *Phytophthora* grown in a dry atmosphere showed retardation of growth and enhanced production of chlamydospores.

(*b*) *Light*

Mycelium was placed in distilled water in watch glasses of which six were placed in bright light against a north window and another set of six in a dark chamber, and was examined at the end of every hour. It was observed that the sporangia that were formed germinated by means of zoospores in the light, but that only direct germination took place in the dark. Light therefore seemed to favour indirect germination through the agency of zoospores. Few chlamydospores and no oospores were observed.

VII. Mixed cultures

As the fungus did not form oospores in cultures, attempts were made to induce oospore formation by growing this *Phytophthora* together with others in the same Petri dishes. First attempts to induce oospore formation in this manner were made by Clinton [1909-10] and since then Gadd [1924]. Lester-Smith [1927], Ashby [1929, 1 ; 1929, 2] and Narashimhan [1930] have succeeded in obtaining oospores by growing various strains in mixed cultures. In the present study, investigation was carried along two lines : (1) paired cultures by growing together various isolates, all of which were monosporangial in origin, from diseased cinchona plants made on various occasions, to see if heterothallism existed : (2) paired cultures using one of the following fungi against one of the cinchona isolates :—*P. colocasiae*, *P. parasitica* from guava, *P. parasitica* from cotton, *P. meadii*, *P. faberi* and *P. palmivora*. In paired cultures using various combinations of the isolates from cinchona seedlings, no oospores were at any time found, indicating that there may not be heterothallism in this fungus at least so far as the isolates that were used in the present study are concerned. In the case of paired cultures using the other *Phytophthora* spp. oospores were obtained in all cases except *P. meadii*. The results are tabulated in Table II, together with extreme and mean measurements.

Table II

Oospore formation in paired cultures of Phytophthora *on Quaker oat agar in Petri dishes*

Nature of isolates grown together	Oospore formation	Measurements of 100 oospores	
		Range in μ	Mean in μ
Cinchona P1 × Cinchona P2 . . .	—	..	..
Cinchona P *X P. colocasiae* . . .	+	23·4—39·6	31·5
Cinchona P *X P. parasitica* (guava) .	+	21·6—34·2	28·2
Cinchona P *X P. parasitica* (cotton). .	+	25·3—36	30·4
Cinchona P *X P. meadii* . . .	—	..	..
Cinchona P *X P. faberi* (cocoa) .	+	23·4—36	29·9
Cinchona P *X P. palmivora* (palm) . .	+	23·4—34·2	29·4*

*Only fifty oospores were available for measurement in this case.

The Petri dishes containing the paired cultures were placed in an incubator registering 22°C. Oospore formation took place in all cases at the place where the mycelium of the two isolates met. It may be mentioned that at ordinary laboratory temperatures, around 28°C., no oospore formation took place.

At the junction of the mycelium from the two fungi, there was usually a pale yellow line and on microscopic examination, it was found that oospore formation at this place was quite profuse. The antheridia were amphigynous, the oogonial wall was smooth, thin and hyaline while the wall of the oospore was rather thick and yellowish. The oospores looked rather yellowish in colour. Attempts to germinate the oospores were negative.

The question arises whether the oospores produced in these mixed cultures were the result of hybridisation or of some stimulus which one fungus provided to the other for their development. Literature on this point has been recently summarised by Tucker [1931], and Narashimhan's [1930] view, that there is heterothallism in the species of *Phytophthora* with which he worked and that the oospores are produced as a result of hybridisation between the + and — strains does not seem to find favour. In the present studies the isolates did not form oospores in pure culture nor in mixed cultures of cinchona strains but only in mixed cultures with different species of *Phytophthora*. The trials are too few, however, to enable one to draw any definite conclusions from these data, but the fact that the oospores were formed only at the junction of the mycelia from the two different cultures, is significant. It may mean either that the oospores are a result of hybridisation or that some stimulus is provided by one culture to the other for oospore formation, which need not be necessarily chemotropic but can also be thigomotropic.

VIII. Identity of the Cinchona *Phytophthora*

Finally the question of the identity of the *Phytophthora* isolated from the diseased seedlings of cinchona remains to be determined. For this purpose several criteria that are usually applied for the determination of species of *Phytophthora* were applied and the results are presented below. But it has to be emphasized here that the question of taxonomy of the species belonging to this genus is extremely difficult. Attempts by Rosenbaum [1917] to monograph the genus and those by Leonian [1925] to classify the species on the basis of physiological responses have not been conclusive. The recent effort of Tucker [1931] has however clarified the ideas regarding the taxonomy of the genus to a considerable extent.

The following criteria for the determination of the specific rank of cinchona *Phytophthora* were tried :—

(*a*) Pathogenicity and cross inoculation experiments.
(*b*) Critical temperature for growth.
(*c*) Ability to infect certain selected hosts.
(*d*) Morphological comparison including measurement of sporangia.

(*a*) *Pathogenicity tests.*—Pathogenicity tests were conducted by testing the parasitism of three species of *Phytophthora* on cinchona seedlings, and by testing the parasitism of the species from cinchona on castor, palm, betel vine and colocasia. Healthy and vigorously growing seedlings of cinchona were obtained for this purpose and they were carefully infected with the respective species using the usual aseptic methods. The results of these tests are given in Table III.

TABLE III

Pathogenicity of certain Phytophthora *spp. to cinchona seedlings*

Name of parasite	No. of cinchona seedlings	No. killed
P. parasitica	14	*Nil*
P. colocasiae	15	,,
P. palmivora	15	7
Cinchona P.	15	15

From the data recorded in Table III, it will be noted that *P. parasitica* and *P. colocasiae* are unable to parasitize cinchona seedlings but that *P. palmivora* can do so. Of the fifteen plants that were infected with this fungus seven died showing the typical symptoms while all the plants infected by the cinchona fungus succumbed within that time. These infection experiments indicate that the cinchona species may be a strain of *P. palmivora*. This point was settled by the following further experiments.

(*b*) *Critical temperature for growth.*—Tucker [1931] considers that the maximum temperature at which the growth of a *Phytophthora* can take place may yield a clue as to its identity. While this is not an absolute criterion, it certainly helps in arriving at a conclusion along with the other criteria. The fungus was grown in Petri dishes on corn-meal agar and placed in incubators that were accurately adjusted to 32·5°, 35° and 37·5°C. None of the species included in this test grew at 37·5°C. At 35°C. *P. parasitica*, *P. colocasiae* and *P. palmivora* grew slightly. The cinchona *Phytophthora* grew only at 32·5° and not at the higher temperature.

That the cinchona *Phytophthora* did not grow at 35°C. when *P. palmivora* did, is possibly due to the fact that while *P. palmivora* was an isolate from a palm tree from the plains, the cinchona *Phytophthora* came from the mountain valleys where it may have, in the course of years, become accustomed to a cooler temperature. Attention may be drawn in this connection to the twenty-two isolations of *P. palmivora* studied by Tucker [1931]. While he considers them all to belong to the same species, they show considerable variation in their response to the same temperature so that the slight variation seen between the cinchona *Phytophthora* and *P. palmivora* in this case is not very material.

(c) *Ability to infect certain hosts.*—In order to ascertain the ability of cinchona *Phytophthora* to infect certain hosts, its parasitism was tried on leaves of castor, colocasia, betel vine and palm. The usual aseptic precautions were taken in these tests and the standard methods of testing were tried. The results are given below:

TABLE IV

Parasitism of cinchona Phytophthora *on certain hosts*

Host	No. infected	No. that took infection
Castor leaves	17	12
Colocasia leaves	12	*Nil*
Betel vine leaves	14	,,
Palm leaves	15	8

The results show that only castor leaves and palm leaves are infected by the cinchona *Phytophthora*. Even though the castor leaves are attacked by the cinchona fungus, the castor fungus, *P. parasitica*, is unable to infect cinchona seedlings. In the case of infection of palm leaves, however, the cinchona fungus is not only able to infect the palm leaves but the palm *Phytophthora* is able to infect cinchona seedlings. It would appear therefore that the *Phytophthora* from cinchona is not *P. parasitica* but a strain of *P. palmivora*.

(d) *Morphological comparison and spore measurements.*—The above conclusion was further supported by the morphological features of the two fungi and their spore measurements. Butler [1910] mentions that the sporangia of *P. palmivora* have a distinct stalk at the base. The sporangia of cinchona *Phytophthora* possess also a distinct stalk. It therefore resembles *P. palmivora* in this respect rather than *P. parasitica* which according to McRae [1934] is without stalks. Furthermore according to Butler the papilla of *P. palmivora* are very prominent. Critical comparison of the papilla of cinchona *Phytophthora* again showed its close resemblance to *P. palmivora* rather than to *P. parasitica*.

For the purpose of comparing the spore sizes, 200 sporangia were measured, both of cinchona *Phytophthora* and *P. palmivora*. The method of obtaining these sporangia is already given in a previous section. The measurements were arranged in the form of frequency arrays, both for length and breadth, and these are given in Table V, together with the mean measurements. For seeing whether there was any agreement between the lengths and breadths of *P. palmivora* and cinchona *Phytophthora*, Pearsons' χ^2 test as applied by Mundkur [1934] was used. The values of χ and P obtained in each case are also given at the end of Table V.

TABLE V

Frequency tables of length and breadth of the sporangia of cinchona Pnytophthora *and* P. palmivora

Mid-point	Cinchona *Phytophthora*		*P. palmivora*	
	Length in μ	Breadth in μ	Length in μ	Breadth in μ
9·5		18		15
13·5		50		35
17·5	1	76	1	56
21·5	20	39	13	60
25·5	36	14	29	27
29·5	28	3	27	6
33·5	42		41	1
37·5	28		35	
41·5	24		29	
45·5	13		11	
49·5	4		10	
53·5	2		1	
57·5	2		3	

Mean length 33·3±·39. Mean length 34·7±·36.
Mean breadth 17·6±·19. Mean breadth 18·9±·23.

Agreement between lengths of sporangia of cinchona *Phytophthora* and *P. palmivora* } $\chi^2 = 6 \cdot 78$.

P for $n = 11$ is between ·80 and ·70 (Fisher's table)

Agreement between breadths of sporangia of cinchona *Phytophthora* and *P. palmivora*. } $\chi^2 = 43 \cdot 79$.

$P < 0 \cdot 01$

It will be noted from the data recorded in Table V that the sporangia of cinchona *Phytophthora* have a slightly smaller mean than those of *P. palmivora* but the mean difference is not however significant as the value of P is high, between ·80 and ·70. With respect of length therefore the two fungi can be considered as same.

With respect to breadth, the sporangia of cinchona *Phytophthora* are slightly narrower than those of *P. palmivora*. The mean difference in this case, however, is significant as the value of *P* is less than ·01. Comparison with the measurement of the sporangia of *P. colocasiae*, and *P. parasitica*, made in connection with some other investigations at the same time as these investigations, showed however that the cinchona fungus agreed closely in respect of sporangial size with *P. palmivora* rather than with the others.

In a recent paper Celino [1934] has described a *Phytophthora* on cinchona from the Philippines and named it *P. faberi* Maubl. When discussing the taxonomy of cinchona *Phytophthora* he states "According to Rosenbaum's tentative table for the separation of the species of the genus *Phytophthora* the absence of oospore places the fungus in the *Faberi* group. The Philippine *Phytophthora*, cinchona strain therefore falls under this category". Reinking [1923] also considered his isolations of *P. faberi* distinct from *P. palmivora* because they did not produce oospores. It is customary to consider isolations from cacao, *P. faberi*, and those from palms, *P. palmivora*, but it is doubtful whether any real difference exists between the two species. Celino [1934] has mainly depended on the size of sporangia and chlamydospores for the classification of his species. Tucker [1931], Leonian [1925] and other workers have shown that the sporangia and chlamydospores of *Phytophthora* of the same species vary so much in size that they cannot be depended upon for specific identification. Butler [1924] and Gadd [1924] considered *P. faberi* and *P. palmivora* morphologically similar, while Leonian [1925] considered them alike physiologically. Lester-Smith [1927], Seal [1928], Ashby [1929], Thomson [1929] and other writers regarded the two species as identical and the majority recognised the validity of *P. palmivora* as the specific name. The writer therefore prefers to name his isolation from cinchona as *P. palmivora* Butl.

IX. Summary

1. A species of *Phytophthora* causes a seedling blight of cinchona in the Sikkim plantation of the Darjeeling district.

2. Inoculation experiments on the stems and leaves of cinchona with the isolates proved the pathogenicity.

3. The optimum temperature for the growth of cinchona *Phytophthora* is found to be about 24°C. Above and below this temperature the growth decreases and at 35°C. there is no growth at all.

4. Cross-inoculations with cinchona *Phytophthora* and *P. palmivora* Butl. on cinchona and palm leaves respectively gave successful results.

5. Morphological comparisons of cinchona *Phytophthora* with *P. palmivora* Butl., *P. meadii* McRae, *P. colocasiae* Rac. and *P. parasitica* Dast. indicated that it agrees most closely with *P. palmivora* Butl.

6. Statistical comparison of the sizes of sporangia of cinchona *Phytophthora* and *P. palmivora* Butl. were made and it was found that in length the sporangia of both the fungi agree but in breadth the sporangia of cinchona *Phytophthora* are slightly narrower than those of *P. palmivora* Butl.

7. It is concluded therefore that the cinchona fungus is a strain of *P. palmivora* Butl.

X. Acknowledgments

The writer wishes to take this opportunity to render thanks to Dr. W. McRae for suggesting the problem and for providing the necessary facilities for conducting the research. Grateful acknowledgments are made of the help given by Dr. B. B. Mundkur in preparing the paper and in aiding in the biometrical analysis, and to Dr. Mitra for making useful suggestions.

REFERENCES

Ashby, S. F. (1929, 1). *Trans. Brit. Myc. Soc.* **14**, 18-38.
———— (1929, 2). *Ibid.* **14**, 260-263.
Butler, E. J. (1910). *Mem. Dept. Agric. Ind., Bot. Ser.* **3**, 1-64.
———— (1924). *Rept. Imp. Bot. Conf. London* 145-147.
Celino, M. S. (1934). *Philip. Agric.* **23**, 111-130.
Clinton, G. P. (1909-10). *Rept. Conn. Agric. Expt. Stn.* 753-774.
Gadd, C. H. (1924). *Ann. Roy. Bot. Gard., Peradeniya* **9**, 47-89.
Keitt, G. W. (1915). *Phytopath.* **5**, 266-269.
Klebs, G. (1899). *Jahr. f. wiss. Bot.* **33**, 513-593.
Leonian, L. H. (1925). *Amer. J. Bot.* **12**, 444-498.
Lester-Smith, W. C. (1927). *Ann. Roy. Bot. Gard., Peradeniya* **10**, 243-257.
McRae, W. (1934). *Ind. J. Agric. Sci.* **4**, 585-617.
Mundkur, B. B. (1934). *Ind. J. Agric. Sci.* **4**, 758-778.
Narasimhan, M. J. (1930). *Phytopath.* **20**, 201-214.
Reinking, O. A. (1923). *J. Agric. Res.* **25**, 267-284.
Rosenbaum, J. (1917). *J. Agric. Res.* **8**, 233-276.
Seal, J. L. (1928). *Florida Agric. Expt. Stn. Bull.* **199**.
Thomson, A. (1929). *Malayan Agric. J.* **17**, 53-100.
Tucker, C. M. (1931). *Missouri Agric. Expt. Stn. Res. Bull.* **153**.

STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB

I. SYMPTOMS, INCIDENCE AND CAUSE OF THE DISEASE

BY

R. SAHAI VASUDEVA, B.Sc., PH.D. (LOND.), D.I.C.,

Assistant Cotton Mycologist, Punjab Agricultural College and Research Institute, Lyallpur

(Received for publication on 5th March 1935)

(With Plates XXI and XXII and two text-figures.)

CONTENTS

I. INTRODUCTORY

The root-rot disease of cotton is very serious in the Punjab and causes heavy damage to the crop every year. It has been prevalent for many years and affects both American and indigenous (*desi*) cottons. The disease attacks the roots exclusively and brings about at first wilting of shoots and then death of the entire plant. The damage caused by the disease to cotton has been found to vary a great deal in different fields. Observations made on the cotton crop in the Sargodha Colony showed that the percentage of damage was 1-15 in different fields; while that on the Lyallpur Agricultural Farm, on the average, the loss has been about 3·3 per cent. In certain individual fields the loss is very great. In some plots of the Botanical area at Lyallpur, about 11 per cent of the

crop was destroyed by the root-rot disease annually. In other plots, however, as much as 30 per cent plants were killed by this disease. In certain fields on the Agricultural Farm, Montgomery, over 50 per cent of the crop had been reported as affected by root-rot.

Incidence of the disease in zamindars' area.—Reports received regarding the occurrence of this disease in zamindar's fields in various parts of the Province have shown that the disease occurs commonly wherever cotton is grown. In some cases, the damage reported has been as great as 60 per cent of the whole crop. Taking into consideration the extent of the disease for the whole province, the average loss, at a moderate estimate may be put at about 2·5 per cent. On this basis the area of the cotton crop damaged by the disease would be 56,250 acres, taking $2\frac{1}{4}$ million acres as the total area under cotton in the Province. If four maunds be taken as the average outturn per acre and Rs. 7 as the price of cotton per maund, the total value of the cotton crop destroyed by the disease would amount to (56,250 acres × 28) = Rs. 15,75,000 ($15\frac{3}{4}$ lakhs) *. This is evidently a very heavy loss. But if the loss of individual fields is calculated, it would come to an alarming figure.

An investigation of the cotton root-rot disease had been carried on by the Punjab Agricultural Department for some time. Preliminary observations made on the diseased roots showed the presence of fungal hyphae and spores and pointed to the occurrence of soil fungi of the order of *Fusarium*. But nothing definite was established regarding the causal organisms. The problem remained unsolved and no further light had since been thrown on the presence of any effective parasitic fungi, as the inoculation of healthy plants failed to produce the disease. The investigation of the problem has been taken in hand under a scheme of the Indian Central Cotton Committee since 1932. The work so far done is briefly described in the paper.

II. Symptoms of the disease

The first and prominent symptom of the disease is the complete wilting of the plant. Early in the morning one can see plants, which have suddenly and completely wilted. From top to bottom every leaf droops down and it is a matter for surprise that plants looking perfectly healthy are killed outright within a day. Sometimes a wilted plant may recover during a cool night, in wet weather or by irrigation, but this is very rare. Usually when once a plant wilts, there is no recovery.

The suddenness of wilting is a characteristic feature of this disease.

Plants affected by root-rot can be easily pulled out of the ground. By removing the soil carefully around the roots of freshly attacked plants, it has been observed that except the tap root and a few secondary roots which hold

* This is a rough estimate and actual survey needs to be made and will be carried out as soon as an opportunity arises.

the plants in the soil, all other roots are decayed and detached. The disease actually starts much earlier and the above-ground manifestation of the disease in the form of wilting is, therefore, a very late symptom. The tip of the root of a freshly wilted plant is slightly moist and sticky to the touch. On a white surface it leaves a yellowish stain. The bark tissue is much shredded. On some freshly wilted plants the yellowing and shredding extends above the terminal portion. Plants that have recently succumbed to the disease have all their root tissues, particularly the bark, broken down into shreds. The central woody cylinder is not affected within but turns golden yellow in colour on the surface. In badly affected plants the wood also becomes brown or black. A plant showing typical symptoms of the root-rot disease is shown in Plate XXI, fig. 1. Plate XXI, fig. 2 shows the root-system of the same plant magnified.

Underneath the bark, there is a deposit of yellowish substance of granular nature. The lumen of the wood vessels is filled in several cases with yellow granular material. The shredded bark is found to contain fungal hyphae and these have sometimes been observed even in the xylem vessels.

Cotton is known to suffer from root-rot in the U. S. A. It is also reported from various other cotton-growing tracts of India. Although the Punjab root-rot of cotton resembles the Texas (U. S. A.) and the Gujerat (India) root-rot in some respects, it has distinct characters of its own. The suddenness of wilting, destruction of the major portion of the roots, yellowing of the tissues and shredding of the bark are common characters. When once a palnt wilts, it seldom recovers and the leaves of plants attacked by root-rot hang down over the branches and remain attached for some time. Peltier [1926] records that in the Texas root-rot, there is bronzing or yellowing of the leaves about 24 hours before wilting. This has not been observed in the Punjab. In Gujerat the sclerotia of *Rhizoctonia* are found in abundance within the bark and the fungus can be readily isolated [Likhite, 1934]. In the Punjab, sclerotia have been observed in rare cases.

In Texas the root-rot is caused by the fungus *Phymatotrichum omnivorum*, originally described by Shear [1907] as *Ozonium omnivorum*, but later assigned to the genus *Phymatotrichum* by Duggar [1916]. It makes its appearance when the squares are being formed and seldom earlier. The Gujerat root-rot is at its height during the flowering and boll developing stages. The Punjab root-rot appears when the plants are about two months old and so is much earlier and almost ceases during the flowering time. Taubenhaus and Killough [1923] mention that the strands of *Phymatotrichum omnivorum*, the causal organism of Texas root-rot are seen covering the epidermis. In the Punjab also fungal hyphae have in certain cases been seen on the surface of the roots. *Phymatotrichum omnivorum*, the Texas root-rot fungus, attacks a great variety of crops. It is still a matter for investigation whether Punjab root-rot fungus can infect other plants.

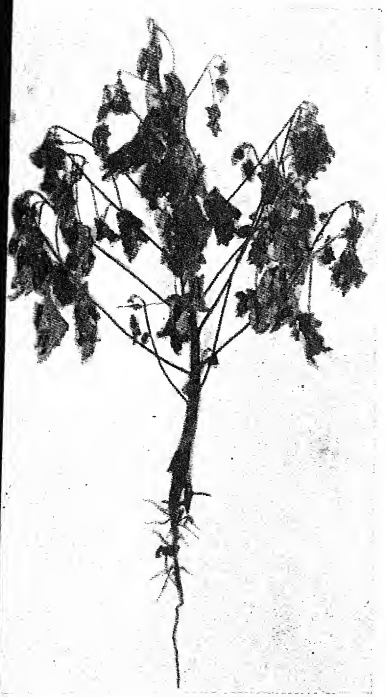


FIG. 1.
A cotton plant affected with root-rot.

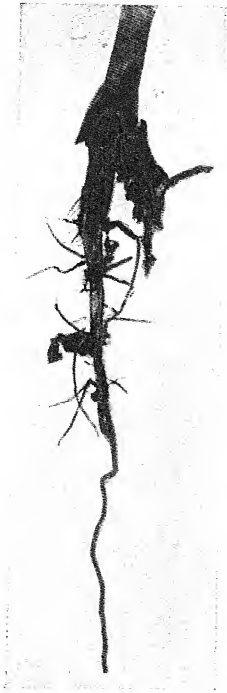


FIG. 2.
Root system of the same plant.

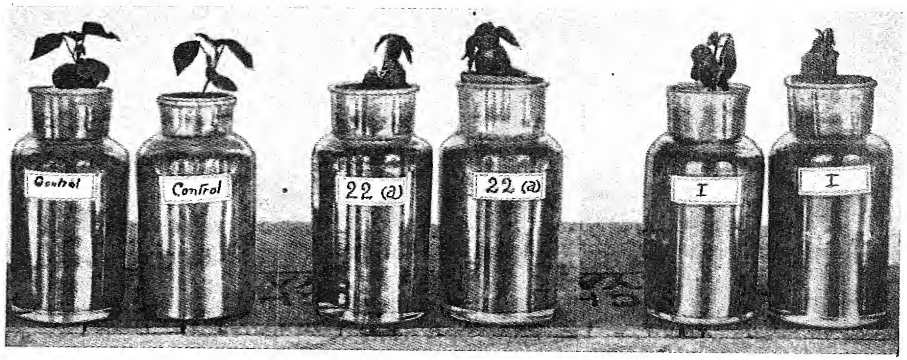


FIG. 3. Cotton plants inoculated by transplanting method with the two species of *Rhizoctonia*, I and 22 (*a*). Control plants are also shown.

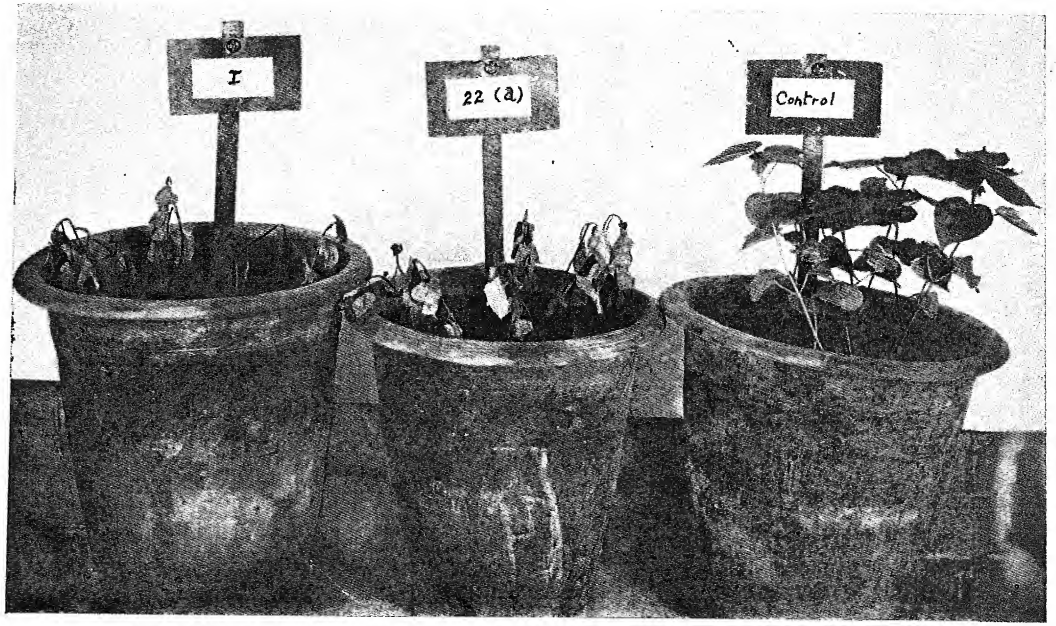


FIG. 4. Wilted cotton plants inoculated with *Rhizoctonia* species I and 22 (*a*) by root washing method along with controls.

III. Development of the disease

With a view to studying the cause of the disease, a number of cotton plants in rows showing different stages of root-rot symptoms along with apparently healthy plants were fastened together by means of cords. The ends of the cords were tied to vertical sticks at each end of the row. Trenches about 5 feet deep were dug on either side of each row and care was taken that none of the lateral roots were damaged. Then the soil was removed around the roots and most of the larger roots were thus exposed for examination in the 2nd week of August. As the plants were tied up, they did not fall over.

The results of the examination of roots thus exposed are given below. The plants showing the effect of root-rot at different stages are grouped separately :—

Group I. (*6 plants*).—All parts healthy; tap root and lateral roots all sound. No fungal mycelium visible on the roots. In two plants, however, slight yellowing of the roots was observed.

Group II. (*2 plants*).—All shoots healthy; tip of the tap root infected and rotting; lateral roots healthy; white mycelial, strands visible on several roots.

Group III. (*2 plants*).—All shoots healthy; tap root rotten about 6-8 in.; brown in colour; soft; bark shredding; lateral roots slightly yellow.

Group IV. (*2 plants*).—Shoots wilted; roots only slightly yellowish.

Group V. (*6 plants*).—Wilted for 2-3 days; leaves drooping.

(*a*) (2 plants).—Tap root soft; yellowish material exuding; tap root infected; lateral roots also slightly yellowish; bark not peeling off.

(*b*) (2 plants).—Tap root infected; brownish in colour about two feet above the tip; yellowish material exuding; tap root rotting and soft.

(*c*) (2 plants).—Tap root rotting; brown and yellowish in places; lateral roots showing symptoms of yellowing.

Group VI. (*8 plants*).—Wilted and dead for 7-10 days; wilted leaves hanging on.

(*a*) (1 plant).—About 12 inches of tap root rotten; bark shredding, yellow to dark brown in colour; bark of the laterals peeling off and some rotting; yellowish liquid exuding from large laterals.

(*b*) (2 plants).—Tap root infected and rotting over 6 inches length; central cylinders brown; bark around this point yellow on the inner side; yellowish material exuding; laterals yellowing and bark peeling.

(*c*) (3 plants).—Whole of tap root (27 inches) rotting and yellow; tip of the tap root brown and infected; lateral roots yellow and soft.

(*d*) (2 plants).—Tap root infected and brown; soft and rotting; laterals and tap root all showing yellowing and shredding.

IV. Incidence of the disease

To study the incidence and progress of the disease from week to week, certain plots in the Botanical area and Agricultural Farm at Lyallpur in which the disease is known to occur year after year were kept under observation for two seasons. The actual position of the infected plants was marked and the rate of progress of the disease was recorded. Similar observations were also made at Hansi and Montgomery.

Observations at Lyallpur indicate that the attack starts when the cotton plants are about six weeks old. The disease first appears in June. Within a fortnight the maximum percentage of attacked plants is reached. It, however, continues to be vigorous throughout July. In August the rate of death of plants gradually declines and the disease almost ceases by the end of September. Figs. 1 and 2 show the progress of the disease in a certain plot at Lyallpur during 1932 and 1933, respectively.

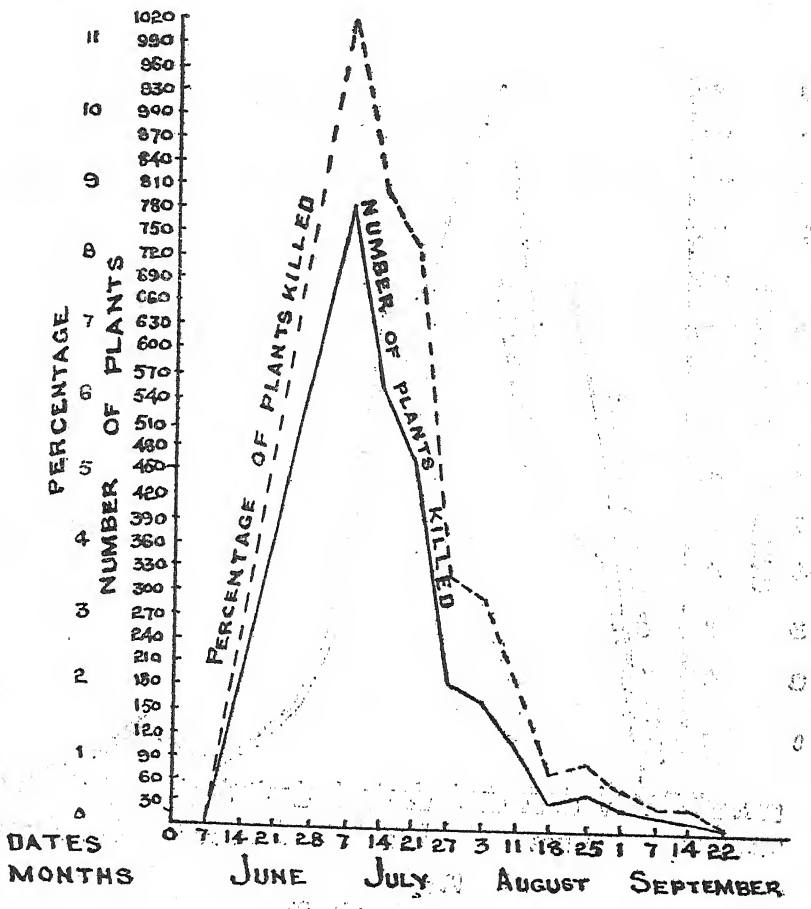


FIG. 1.

Square 46, plot 23

Mortality of plants due to root-rot, 1932

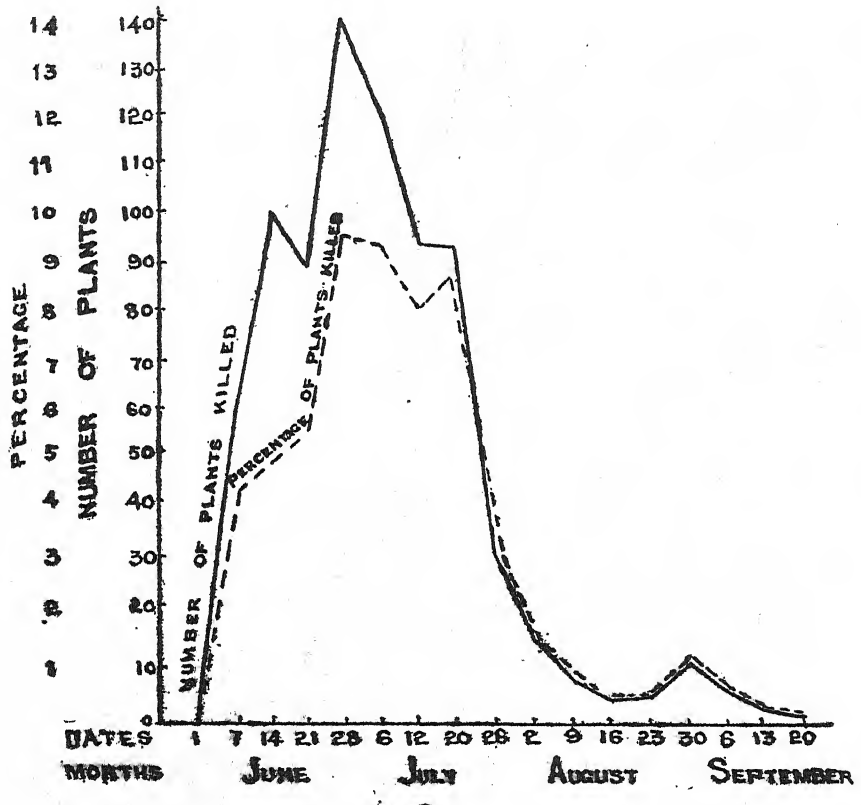


Fig. 2

Square 46, plot 23

Mortality of cotton plants due to root-rot, 1933

Similar observations were made at Hansi and Montgomery. The results obtained at Hansi were identical with those of Lyallpur. But at Montgomery the disease appeared a little later and the optimum attack was delayed till the first week of August. Table I shows the mortality due to root-rot in some severely affected plots under observation at Lyallpur and Montgomery in 1933.

TABLE I

Mortality due to root-rot at Lyallpur and Montgomery in some severely affected plots

Date of observation		Lyallpur			Montgomery		
Month	Week	Total No. of plants	No. of plants killed	Per cent attack	Total No. of plants	No. of plants killed	Per cent attack
June	1st	1,677	63	3·76	..	..	..
	2nd	1,614	102	6·31	..	..	..
	3rd	1,512	93	6·17	..	..	..
	4th	1,419	138	9·72	1,067	21	1·96
July	1st	1,281	120	9·37	1,046	79	7·55
	2nd	1,161	95	8·18	967	85	8·79
	3rd	1,066	93	8·73	882	89	10·09
	4th	973	35	3·60	793	89	11·22
August	1st	938	17	1·81	704	90	12·78
	2nd	921	9	0·97	614	65	10·59
	3rd	912	4	0·44	549	38	6·92
	4th	908	6	0·66	511	30	5·87
September	1st	902	13	1·44	481	16	3·33
	2nd	889	7	0·79	465	12	2·58
	3rd	882	2	0·23	453	8	1·77
	4th	880	0	0	445	6	1·35

It is clear from the above that in the plot under observation at Montgomery, out of the total of 1,067 plants at the beginning of the season, 632 succumbed to the disease showing a loss of 59·2 per cent. At Lyallpur the damage was 47·5 per cent. These two examples illustrate the ruinous damage in severely affected areas.

V. ISOLATION OF THE CAUSAL ORGANISM

In order to isolate causal organisms, material from different parts of the root system of the diseased plants was cultured. The plants that had wilted as a result of the disease were dug up and the earth washed off the roots. Small

pieces of tissues were dipped in mercuric chloride (1/1000) solution for 30-60 seconds. These were then given a thorough washing with sterile distilled water and transferred to Petri dishes containing plain agar. As soon as the mycelium grew from the transfers, inocula from these were planted on Richards' agar. For these isolations, diseased plants were obtained from various localities, *i.e.*, Lyallpur and neighbouring villages, Hansi, Sirsa, Montgomery, Sargodha and Khanewal. The total number of isolations from time to time went up to several thousands. The fungi thus reproduced were examined and it was observed that two species of *Rhizoctonia* appeared most frequently. For convenience these fungi will be referred to as I and 22 (*a*). In addition, two strains of a *Fusarium* appeared frequently, either alone or associated with *Rhizoctonia sp.* Besides *Rhizoctonia sp.* and *Fusarium*, a species of *Alternaria* and *Helminthosporium* appeared occasionally. Some saprophytic fungi were also found.

When the wilted plants were kept in a moist chamber overnight, a cottony white mycelium broke through the root tissue and appeared on the surface in abundance. This fungus was also found to be one of the above-mentioned *Rhizoctonia sp.*

It may be mentioned that Butler [1918] also isolated *Rhizoctonia* from roots of cotton plants attacked by root-rot in North-Western India.

VI. Identification of the fungi

Rhizoctonia sp. I is a strain of *Rhizoctonia solani* Kuhn group. It seems to differ from all other strains described so far.

Rhizoctonia sp. 22 (*a*).—This fungus is rather problematical. The scelrotial and hyphal characters are much like those of *Rhizoctonia bataticola* but no strain of it is known which produces so much white aerial mycelium. It is suggested that it may be called *Rhizoctonia bataticola* provisionally, as it is perhaps a saltant.

The two *Fusaria* (A and B) that were isolated have also been identified and are strains or 'Forms' of *Fusarium solani* (Mart.) App. and Wr. In the case of strain B, macroconidia are in definite sporodochia. Strain A does not produce macroconidia in definite sporodochia but is mixed with microconidia on the aerial mycelium. Moreover, macroconidia of strain A are broader than those of strain B.

VII. The causal organism

The various fungi isolated were tested for their parasitism on cotton plants of different ages growing in the fields as well as in pots under field and controlled laboratory conditions. Inoculations were made by various methods, which are discussed fully elsewhere. In Table II are given results of one of the typical sets of experiments carried out with plants in pots.

TABLE II

Results of inoculations carried out in pots with different fungi

Cotton plant		Inoculated with *Rhizoctonia sp.* I			Inoculated with *Rhizoctonia sp.* 22 (*a*)			Inoculated with *Fusarium* A			Inoculated with *Fusarium* B		
									No. infected			No. infected	
Variety	Age (week)	No. inoculated	No. infected root-rot	Per cent infection	No. inoculated	No. infected root-rot	Per cent infection	No. inoculated	Root-rot	Other causes	No. inoculated	Root-rot	Other causes
4-F . .	3	11	1	9·09	12	11	91·66	12	0	0	10	0	0
Mollisoni .	4½	10	2	20·00	12	10	83·30	11	0	6	9	0	2
„ .	10	13	5	38·46	6	2	33·33	7	0	0	6	0	0
„ .	11	13	2	15·38	6	5	83·33	7	0	0	7	0	0
„ .	12	9	2	22·22	6	3	50·00	7	0	0	7	0	0

It is clear from Table II that *Rhizoctonia sp.* I and 22 (*a*) reproduce the disease. In the case of *Fusarium* A and B wilting of inoculated cotton plants once obtained was not typical of root-rot and other characteristic symptoms, *e.g.*, shredding and peeling off of root bark were also absent.

All the remaining fungi isolated were also included in the above pathogenicity tests, but they failed to produce infection or any symptoms of the disease, Throughout these experiments controls were kept side by side, *i.e.*, the plants were given exactly the same treatment as the ones inoculated, with the only exception that no fungal inoculum was added.

As the two species of *Rhizoctonia* appeared most frequently in isolations from diseased material and successfully reproduced the disease, these were selected for further study.

VIII. Material and methods

Only two varieties of cotton, namely, Desi *G. indicum* var. (*Mollisoni*) and American (4-F) were used for inoculation purposes throughout the investigation. The plants were raised in pots, containing sterilized soil, kept under normal field and controlled laboratory conditions. For field inoculation experiments, the crop was grown in rows 3 feet apart in plots, where root-rot disease had not appeared previously. During winter season the plants were grown in jars kept in hot water tanks maintained at the desired temperature, glass house and a special glass-walled chamber, which was fitted with an electric thermostat and heating bulb to provide the necessary temperature. During exceptionally cold nights the chamber was covered with felt blankets. The chamber was kept in a verandah where plenty of bright diffused light was available to the plants. For inoculation purposes both mass infection and single plant infection were tried. The source of inoculum was added either in the form of rots of freshly wilted plants due to root-rot, or pure cultures of the fungi, growing on sterilized* cotton roots, paddy or nutrient agar.

IX. Infection experiments

Preliminary infection experiments were carried on both in the field and in pots with all the fungi that had been isolated.

Two methods were employed for the infection experiments in the field.

(1) individual plant inoculation and

(2) mass inoculations.

Inoculations were made by direct infection (using the roots of diseased plants as the source of inocula) and pure culture infection methods.

*To obtain thorough sterilization it was found necessary both in the case of cotton roots and paddy to give three 20-minutes steam-sterilizations at 15 lbs. pressure on alternate days.

The roots of plants were exposed carefully to a depth of about eight inches and the inoculum was placed parallel to the tap root and in places in actual contact with laterals. Sterile moist cotton wool was applied with the inoculum to keep it moist. The soil was replaced and the plants were watered freely. Controls were treated similarly except that no inoculum was added.

In the preliminary inoculation experiments, tests were made with the different fungi isolated, but none of the plants developed any obvious signs of root-rot. In a few cases, however, where the plants were inoculated with either of the *Rhizoctonia sp.*, though they had perfectly healthy appearance, some of the roots were affected and showed characteristic shredding of the bark tissue. From such roots the *Rhizoctonia sp.* were readily isolated. These experiments were carried out late in the season and possibly the plants had outgrown the stage of infection.

Infection experiments were repeated with plants growing in pots using pure fungal cultures on nutrient agar as source of inoculum, but without success.

A number of further preliminary experiments were conducted, but the methods of inoculation were modified in a number of ways in order to eliminate certain factors which might be responsible for the failure of these infection trials. These experiments will now be described.

In the normal method of inoculations the fungal inoculum was introduced near the root and covered up with soil and watered. Under these conditions it was thought that accumulation of carbon dioxide or scarcity of oxygen might be the cause of failure to reproduce the disease. These factors were, however, ruled out by the observation that no attack took place when openings were made in the soil and the fungus was introduced close to the roots and those openings maintained and plugged with sterile cotton wool.

Transplanting method.—Cotton plants were grown in pots containing sterilized soil and when they were of the required age they were removed from these pots by washing out the soil with a fine but powerful jet of water, taking care so as not to injure the roots. These were then given a thorough washing in sterile distilled water and transplanted singly in glass jars containing sterile soil. The jars were fitted with corks having a hole (one cm. in diameter) in the centre and with one radial slit. The plants were put through these slits quite easily without injuring any of the roots. Sterile water was then added and the jars kept in a glass house during the summer and in hot water tanks (30-33° C.) during winter months. After about a week to ten days when the plants had established their roots, the soil from the top was removed carefully by means of a scalpel, inoculated with pure culture of the fungus on paddy and covered up with the soil that had been previously removed. Controls were treated similarly but in place of infected paddy, only sterilized paddy was introduced. The transplanted plants that did not look healthy were discarded before inoculation.

In another series of experiments the plants were transplanted into glass bottles that contained soil infected with cultures of different fungi on paddy.

Results of four inoculation experiments carried out by transplanting method are given in Table III. During all these experiments, plants of *Mollisoni* variety of cotton were used.

TABLE III

Results of inoculations carried out by transplanting method with different fungi

Date of		Age of plants in weeks	Inoculation with *Rhizoctonia sp.* I.			Inoculation with *Rhizoctonia sp.* 22 (*a*).			Inoculation with *Fusarium* A		Inoculation with *Fusarium* B	
Inoculation	Observation		No. inoculated	No. infected	Percentage infection	No. inoculated	No. infected	Percentage infection	No. inoculated	No. infected	No. inoculated	No. infected
28th Nov. 1932	5th Dec. 1932	8	4	4	100·0	4	2	50·0	4	0	4	0
10th Dec. 1932	15th Dec. 1932	10	6	3	50·0	4	0	0·0	5	0	4	0
5th April 1933	8th April 1933	6-7	5	5	100·0	5	3	60·0	4	0	3	0
21st April 1933	1st May 1933	7	4	4	100·0	3	2	66·6	2	0	3	0

Plate XXI, fig. 3 shows plants which had been transplanted and later inoculated with *Rhizoctonia sp.* I and 22(*a*). The inoculated plants showed wilting, whereas the checks looked healthy and remained sound.

The plants inoculated with the two *Rhizoctonia species* produced characteristic root-rot disease.

Inoculations with the two forms of *Fusarium solani* and species of *Helminthosporium* and *Alternaria* were unsuccessful. Controls always remained healthy.

In the first two experiments in Table III, the plants were transplanted and allowed to establish their roots and then inoculated. In the remaining two experiments the plants were transplanted directly into infected soil.

Root washing method.—In another series of experiments roots of the cotton plants of different ages growing in pots were exposed by washing off the soil around the roots up to a depth of about four inches. The fungus grown on paddy was then introduced, covered up with sterile soil and watered freely. Controls were treated similarly except that no inoculum was inserted. As far as possible aseptic conditions were maintained throughout the course of these experiments. Results of fourteen such inoculation experiments are given in Table IV.

TABLE IV

Results of inoculations carried out in pots by root washing method

Cotton plants		Inoculated with *Rhizoctonia sp.* I			Inoculated with *Rhizoctonia sp.* 22 (*a*)			Inoculated controls	
Variety	Age (weeks)	No. inoculated	No. infected	Per cent infection	No. inoculated	No. infected	Per cent infection	No. of controls	No. infected
Mollisoni	2	23	2	8·70	20	3	15·00	11	All healthy.
4-F	3	11	1	9·09	12	11	91·66	9	,,
4-F	3½	13	3	23·07	11	9	81·81	11	,,
Mollisoni	3½	14	11	78·57	15	5	33·33	12	,,
,,	4½	10	2	20·00	12	12	100·00	15	,,
4-F	5½	11	2	18·18	10	1	10·00	11	,,
Mollisoni	6	29	5	17·24	37	7	18·91	17	,,
,,	7	56	17	30·35	49	12	24·49	33	,,
,,	8	17	3	17·63	14	13	92·85	15	,,
,,	8	12	1	8·33	23	5	21·73	12	,,
,,	9½	15	3	20·00	15	5	33·33	13	,,
,,	10½	13	5	38·46	6	2	33·33	4	,,
,,	11	13	2	15·38	6	5	83·33	6	,,
,,	...	9	2	22·22	6	3	50·00	6	,,

Plate XXI, fig. 4 shows the wilted plants inoculated with *Rhizoctonia sp.* I and 22 (*a*) along with controls. Re-isolations from the infected plants yielded fungi with which they were inoculated.

In further experiments along these lines, the pots were replaced by tin cans with removable double bottoms, so that the roots for inoculation purposes could be exposed by washing from underneath without disturbing the plants. Results of two such experiments are summarised below :—

Fungus	Total plants inoculated	Total infected	Per cent infection
Rhizoctonia sp. I	23	4	17·39
Rhizoctonia sp. 22 (*a*)	27	16	59·26
Controls	30	0	0

Soil infection.—Sterilized soil was infected thoroughly with pure cultures of the fungi. Pots were filled with this soil and cotton sown in these. In the case of controls, no infection was added. Good germination and growth was obtained in control pots as well as those in which the soil had been infected with *Rhizoctonia sp.* 22 (*a*), while in the case of *Rhizoctonia sp.* I, the seed failed to germinate. Such seeds were found to be rotting and *Rhizoctonia sp.* I was readily isolated. Some germination, however, was obtained on third attempt at re-sowing of these pots. Plants in these pots were kept under observation for over three months and throughout this period, plants of different ages wilted from time to time and produced typical root-rot symptoms.

The fungus originally introduced was isolated.

To overcome the difficulty of poor germination due to rotting of the seed, the method of soil infection was modified as follows :—

The pots were filled to one-third of their depth with sterile soil, a layer about three inches in thickness of soil infected with pure cultures of the fungi was added and the rest of the pot filled with sterile soil. The object in view was that the seedlings should get a start before their roots passed into the infected zone.

Inoculations along these lines were carried out on a large scale and the disease was successfully reproduced in the pots which contained soil infected with the two *Rhizoctonia* species whereas the controls remained healthy.

Inoculation experiments in the field.—Trenches about three feet deep were dug along the rows of plants to be inoculated, taking care so as not to injure the roots. The roots of these plants were exposed by washing with a powerful spray hose. Pure cultures of the fungi grown on paddy were then introduced close to the roots and the trenches filled up with the soil that had been previously removed. The roots of plants in control rows were similarly exposed and covered up, but no fungal inocula were added.

To ensure that the wilting was due to the fungi with which the inoculations were carried out, re-isolations were made frequently and the fungi introduced were recovered.

Inoculation experiments in the field were carried out both with American (4-F) and Desi (*Mollisoni*) cottons using plants of varying ages. Results of five such experiments are given in Table V, which shows that the two species of *Rhizoctonia* are highly parasitic in nature.

TABLE V

Results of inoculation experiments carried out in the field with the two species of Rhizoctonia

Experiment	Inoculated with	No. inoculated	Variety	No. infected: Days after inoculation 2	3	4	5	6	7	8	9	10	11	12	13	14	Total infected	Per cent infection
I	*Rhizoctonia sp.* I	21	*Mollisoni*	1	..	4	1	2	..	..	..	..	..	..	1	..	9	42·85
	Rhizoctonia sp. 22 (*a*)	18	(7 weeks)	2	3	2	..	..	..	..	..	..	..	..	..	..	7	38·88
	Controls	19		ALL SOUND													0	0·00
II	*Rhizoctonia sp.* I	44	*Mollisoni*	9	16	2	..	..	1	..	..	..	1	..	..	..	29	65·90
	Rhizoctonia sp. 22 (*a*)	67	(7½ weeks)	..	7	2	..	..	2	..	..	..	..	..	..	..	11	16·41
	Controls	44		ALL SOUND													0	0·00
III	*Rhizoctonia sp. I*	26	*Mollisoni*	1	..	16	..	12 recovered						..	..	..	5	19·23
	Rhizoctonia sp. 22 (*a*)	43	(9 weeks)	3	..	2	3	5 recovered						..	..	..	3	6·97
	Controls	16		ALL SOUND													0	0·00
IV	*Rhizoctonia sp.* I	10	4-F	3	1	..	..	..	..	..	..	..	..	..	..	..	4	40·00
	Rhizoctonia sp. 22 (*a*)	9	(12½ weeks)	..	2	..	..	..	..	..	..	..	..	..	..	..	2	22·22
	Controls	10		ALL SOUND													0	0·00
V	*Phizoctonia sp.* I	7	4-F	..	..	2	1	..	..	..	..	..	..	..	..	..	3	42·85
	Rhizoctonia sp. 22 (*a*)	8	(15½ weeks)	..	..	1	..	..	..	..	..	..	..	..	..	..	1	12·50
	Controls	7		ALL SOUND													0	0·00

Plate XXII, fig. 1 shows rows of plants which had wilted as result of inoculations with *Rhizoctonia solani* (I) and *Rhizoctonia bataticola* 22 (*a*). The rows of control plants are seen on the extreme right.

Plate XXII, fig. 2 shows an entire plant which had wilted due to inoculation with *Rhizoctonia solani* (I). Affected roots of the same plant are shown in Plate XXII, fig. 3.

Inoculations with *Rhizoctonia bataticola* 22 (*a*) produced similar symptoms.

The author wishes to thank Professor Rai Sahib Jai Chand Luthra for the keen interest and helpful suggestions throughout the course of this work. Thanks are also due to Mr. S. F. Ashby of the Imperial Mycological Institute, Kew, for help in the identification of the fungi.

X. Summary

1. Cotton root-rot is a very serious disease in the Punjab. The damage caused is estimated at Rs. 15,75,000 annually.

2. Symptoms of the disease are described.

3. The disease first appears in June and continues to be vigorous during July. In August the attack slows down and almost ceases by the end of September.

4. Several organisms were isolated from the diseased plants and inoculation experiments carried out in the pots as well as in the field by various methods. *Rhizoctonia solani* and another fungus, which is provisionally called *Rhizoctonia bataticola*, have been proved to be the causal organisms.

References

Butler, E. J. (1918). 'Fungi and Diseases in Plants', Calcutta.

Duggar, B. M. (1916). *Ann. Mo. Bot. Gard.* **3**, 11-23.

Likhite, V. N., and Kulkarni, V. G. (1934). *Current Sci.* **3**, 252-254.

Peltier, G. L., King, C. J. & Samson, R. W. (1926). *U. S. Dept. Agric. Bull.* No. **1417**.

Shear, C. L. (1907). *Bul. Torrey Bot. Club* **34**, 305-417.

Taubenhaus, J. J. & Killough, D. T. (1923). *Texas Agric. Expt. Stn. Bull.* No. **307**.

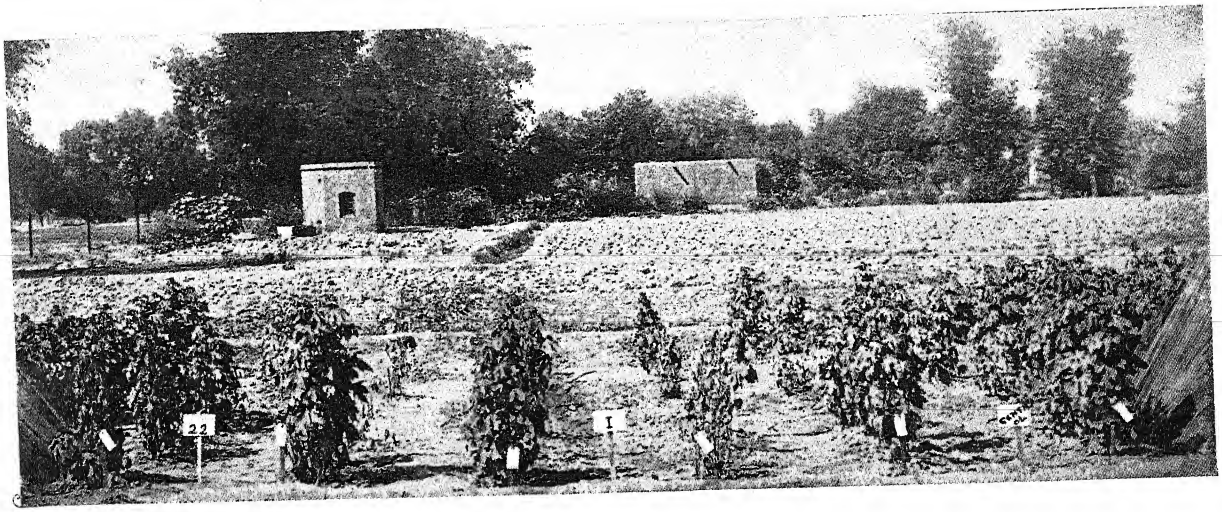


FIG.1. Rows of cotton plants which had wilted as a result of inoculation with *Rhizoctonia* species I and 22 (*a*). Two rows of control plants are seen on the right.

FIG. 2. A cotton plant which had wilted on being inoculated with *Rhizoctonia* sp. I.

FIG. 3. Roots of 4 F cotton plant inoculated with *Rhizoctonia* sp. I.

STUDIES IN INDIAN CHILLIES

(4) INHERITANCE OF PUNGENCY IN *CAPSICUM ANNUUM* L.

BY

RAMCHANDRA BALWANT DESHPANDE, M.Ag. (Bom.),

Offg. Special Research Assistant to the Imperial Economic Botanist, Pusa

(Received for publication on 28th June 1934)

(With Plate XXIII)

CONTENTS

I. Introduction

The property for which the chilli crop is principally grown in India is the pungency of the pods. This pungent property is due to the presence in the fruit of a substance, called capsicin.

Pungency according to our investigations and those of Webber [1911] is definitely a heritable character. The degree of pungency, however, is found to vary considerably with soil and climatic factors. Erwin [1932] also maintains the view that the soil and climate considerably influence the degree of pungency of the chilli pod.

With regard to the mode of inheritance of this important character the results of the investigations of Webber [1911] show that the inheritance of this character is determined by a single factor. These results agree with our observations which also indicate that this character is monogenic.

II. Material and method

For the purpose of these investigations two Pusa types isolated by Shaw and Khan [1928], and since maintained pure, were selected as parents. Type 14 is

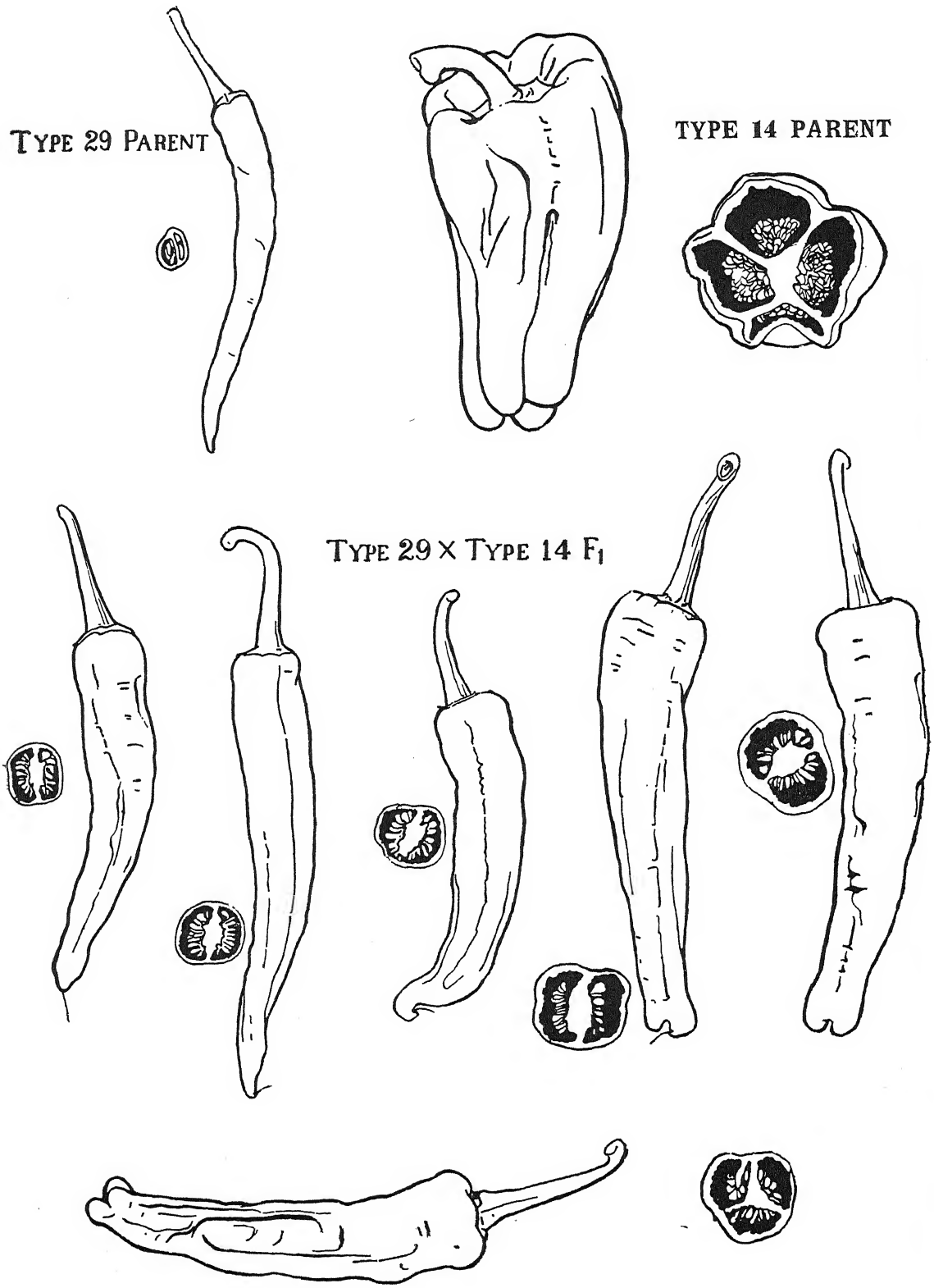
non-pungent while type 29 is pungent. The criterion of pungency was observation by taste by four independent workers. The crosses were attempted in the year 1928-29. A few fruits were obtained with type 29 as female parent but the reciprocal cross failed, and even in subsequent years this reciprocal cross was not successful.

The morphological characters in which the two parents differ are (1) the calyx enclosing or not enclosing the fruit-base, (2) the number of locules in the fruit, and (3) the fruit-apex, lobed or pointed (Plate XXIII). The inheritance of the first character had previously been studied in detail [Deshpande, 1933] in another cross and the character found to be monogenic, 'calyx not enclosing fruit-base' being dominant to 'calyx enclosing fruit-base'. This character in the present cross was found to behave in the same way as in the previous cross and hence is not dealt with here at length but has been considered with pungency to see if any linkage exists between these two characters. The other two characters showed variation in the same plant in F_1 (Plate XXIII) and F_2 and their inheritance study had on this account to be dropped. Similar variation was observed in the previous cross [Deshpande, 1933] in the number of locules in fruits of the same plant in F_2 on which account this character was not further studied.

In F_2 and F_3 the plants were classified into two groups only, as 'pungent' or 'non-pungent' by actually tasting the fruits, though various degrees of pungency were suspected. This method was very trying and slow and it was impossible to examine more than about a dozen plants at a time. The free use of water and cane sugar helped to remove the pungent taste from the mouth but after tasting a very pungent fruit it was never possible immediately to diagnose correctly a less pungent fruit as 'pungent' or a sweet fruit as 'non-pungent.' The doubtful cases had to be gone over again. In order to reduce the personal error the "tasting" was performed by four members of the staff and each diagnosis of pungency was made on these four independent observations. Due to these difficulties, it was not possible to examine the entire F_2 population, which numbered over 500 but we had to be limited to about 270 plants—which, for a monohybrid segregation, is a sufficiently big population. When this population was finally examined and classified the fruits of a few 'pungent' and 'non-pungent' plants were plucked at random and given for tasting to people who had not taken any part in the classification of the F_2 population. In every case the original diagnosis was confirmed. In F_3 all the plants of all the different cultures were examined.

III. F_1 GENERATION

The F_1 generation was grown in the year 1929-30 and consisted of 16 plants. The fruits of these plants were pungent but decidedly less so than the fruits of the pungent parent, type 29, thus proving that pungency is dominant (partially) to non-pungency. Out of these sixteen F_1 plants six were taken at random and selfed to grow the F_2 generation.



Variation in the fruit-apex and the number of locules in the same F_1 plant

IV. F_2 GENERATION

The F_2 generation was grown in the year 1930-31 and had a total population of over 500 of which the first 272 plants were examined and classified. The results are as follows :—

——	Pungent fruited plants	Non-pungent fruited plants	Total
Total observed.	202	70	272
Total expected on 3 : 1 basis . .	204	68	272
Ratio observed	2·97	: 1·03	
Ratio expected	3	: 1	

$$\frac{\text{Dev.}}{\text{P. E.}} = \frac{2}{4\cdot81} = 0\cdot416.$$

The fit, therefore, is very good.

V. THEORY

The above monohybrid segregation can be explained by assuming that **C** is the factor which is responsible for the production of pungency (Capsicin) in the chilli fruit and that when **C** is absent the fruit remains sweet or non-pungent.

The genetic constitution of the parents and the F_1 should, therefore, be as follows :—

Type 29 **CC**—Pungent

Type 14 **cc**—Non-pungent

Type 29 × Type 14 F_1 **Cc**—Pungent (partially)

The F_2 segregation should be on the mono-hybrid basis as follows :—

CC	**Cc**	**cc**
1 :	2 :	1
3 Pungent	:	1 Non-pungent

This result is realized in F_2

VI. F_3 GENERATION

The F_3 was grown in the year 1931-32. In all twenty F_2 plants were carried to the F_3 generation, and the behaviour of these in F_3 was as follows :—

No. of cultures in F_3 and nature of parent plant in F_2	Constitution of F_2 genotypes	Breeding behaviour in F_3	Frequencies	
			Observed	Expected
15 cultures from pungent fruited plants . .	**CC**	Pure	5	5
	Cc	Pungent Non-pungent 3 1	10	10
5 cultures from non-pungent fruited plants . .	**cc**	Pure	5	5

The F_3 observations, therefore, confirm the F_2 segregation on a mono-hybrid basis.

VII. INHERITANCE OF PUNGENCY AND THE NATURE OF CALYX, ENCLOSING OR NOT ENCLOSING FRUIT-BASE WHEN BOTH ARE CONSIDERED TOGETHER ON DI-HYBRID BASIS.

It was ascertained in the previous investigations [Deshpande, 1933] that this calyx character behaves as a simple Mendelian character, 'calyx not enclosing fruit-base' being dominant to 'calyx enclosing fruit-base' and the F_2 observations in the present study confirmed the original results.

Considering the segregation of this character together with pungency, which also is a mono-genic character, we get the following di-hybrid segregation in F_2 :—

	Fruit pungent, calyx not enclosing fruit-base	Fruit pungent, calyx enclosing fruit-base	Fruit non-pungent, calyx not enclosing fruit-base	Fruit non-pungent, calyx enclosing fruit-base	Total
Total observed . . .	134	49	53	17	253
Total expected on 9 : 3 : 3 : 1 basis	142·2	47·4	47·4	15·8	252·8
Ratio observed . . .	8·84	: 3·10	: 3·36	: 1·08	
Ratio expected . . .	9	: 3	: 3	: 1	

$X^2 = 1{\cdot}27$ and $P = 0{\cdot}739$

The fit, therefore, is good.

The above results clearly show that these two characters, pungency of fruits and calyx enclosing fruit-base, both of which have been contributed by one of the parents, type 29, are not linked but segregate independently.

VIII. SUMMARY AND CONCLUSION

(1) Pungency is definitely a heritable character. The degree of pungency, however, is found to vary considerably with environment such as soil, climate, manurial treatment.

(2) The genetic results show that pungency is a simple monogenic character dominant to non-pungency and is determined by a single factor which we have termed **C**.

(3) 'Calyx not enclosing fruit-base' has been found to be dominant to 'calyx enclosing fruit-base' on a 3 : 1 basis.

(4) Both pungency and the nature of calyx, when studied together, have been found to segregate on di-hybrid basis each quite independently of the other.

REFERENCES

Deshpande, R. B. (1933). *Ind. J. Agric. Sci* **3**, 219-300.
Erwin, A. T. (1932). *Iowa Agric. Expt. Stn. Bull.* No. **293**.
Shaw, F. J. F. and Abdur Rahman Khan (1928). *Mem. Dept. Agric. Ind., Bot. Ser.* **16**, No. 2.
Webber, H. J. (1911). Amer. Breeders' Assn. Rept. **8**, 189-199.

PHYLLODY: A POSSIBLE VIRUS DISEASE OF SESAMUM

BY

B. P. PAL, M.Sc., PH.D. (CANTAB.), F.L.S.,
Second Economic Botanist,

AND

PUSHKAR NATH, M.Sc. (HONS.),

Imperial Institute of Agricultural Research, Pusa

(Received for publication on the 18th May 1935)

(With Plates XXIV-XXVII)

The term phyllody is applied to a condition in plants wherein the floral organs are transformed into leaf-like structures. Phyllody is present every year to a greater or lesser extent in *til* (*Sesamum indicum* DC.) at Pusa and in some years as much as 15 per cent of the crop may be affected. As phylloid flowers do not set seed, phyllody, if present in appreciable proportions, may materially lower the yield.

LITERATURE

That phyllody may be a disease of *til* seems to have been suspected as early as 1928 by Robertson [1928] in Burma where it is known as the "Phothe" or "green flowering" disease. He stated that in certain districts as much as 80 to 90 per cent of the plants may be affected, the symptoms manifesting themselves only in the flowering stage when the floral organs are changed into green leaf-like structures, accompanied by abnormally abundant branching.

Deighton [1931] observed a chlorosis attended by mottling and curling of leaves together with enations on the lower surface and inhibition of flowering in *Sesamum radiatum*, and Wallace [1933, 1934] reported a "leaf curl" which he considered to be a virus disease on *S. indicum*, but it is doubtful if the disease was phyllody. Storey [1933] whilst presenting no proof, considered the "green-flower" disease of *simsim* (*S. indicum*) to be also a virus disease but that he was referring to phyllody is apparent from the appropriate name by which the disease is designated. The "sepaloidy" in *til* reported by Kashi Ram [1930] and Roy [1931] is also, in all probability, phyllody. The former attributed the occurrence of the phenomenon to disturbed physiological conditions induced by early sowing and heavy rainfall. The second writer has not hazarded any opinion as to the cause of the abnormality.

Morphology

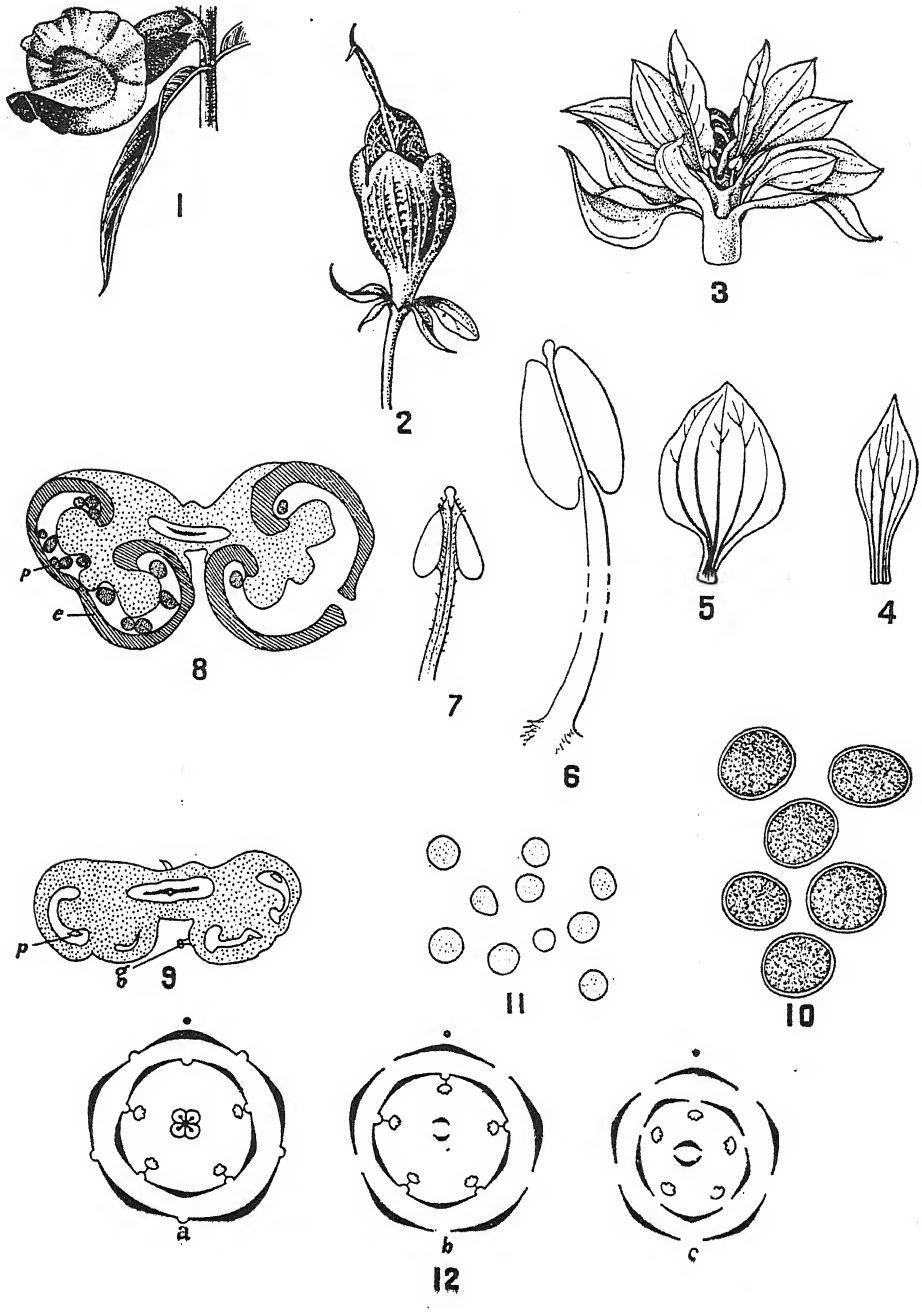
Affected *til* plants bear flowers wherein all the floral members except the stamens are either transformed into leaf-like organs or show a definite tendency to become leafy (Plate XXIV, figs. 2 and 3). The stamens retain their normal configuration but rarely contain functional pollen. In consequence of these changes the fertility of the plant is reduced and complete sterility may result.

The present observations are based on two *til* crops raised in the Botanical Section at Pusa in 1933 and 1934 respectively. Phylloid plants cannot be distinguished from normal ones with certainty until flowering begins, *i.e.*, about 4-5 weeks after sowing in early varieties, and 5-7 weeks in late varieties. Phyllody may begin with the first flower in which case all subsequent flowers on the plant are phylloid or the change may occur later in the ontogeny in which event the first-formed flowers will be normal. In the latter type, phyllody manifests itself simultaneously at the tips of the main axis and the branches and in the new growth from the base so that it is not uncommon to see plants which bear capsules in the middle region but are phylloid at the extremities and at the base. No cases were encountered, however, in which some branches of the plant bore normal flowers exclusively and other branches, phylloid flowers; or in which the first formed flowers were phylloid and later ones normal.

Phyllody is always accompanied by a shortening of the upper internodes so that the abnormal flowers are crowded together and by a diminution in the size of the foliage leaves (in the floral region they are often lighter in colour); the zygomorphy which is well marked in the normal flower is lost and the phylloid flower, whether gamopetalous or polypetalous, is radially symmetrical. Glandular hairs are present on almost all parts of the sesamum plant but in the case of plants exhibiting phyllody these are found to occur as well in regions where they are normally absent, *i.e.*, the filaments and connectives of stamens, the style, stigma, inner surface of carpellary wall, and ovules. In the case of varieties which normally develop only one flower in the axil of each leaf (some varieties bear 2-3), the orange-yellow glands present on either side of the base of the flower stalk are replaced by small and phylloid, lateral, flowers, resulting in three flowers per axil.

The principal changes occasioned in the different parts of the flower, in phyllody, are briefly described below :—

Calyx (*Plate XXIV, figs. 1-4*).—The normal calyx is green, gamosepalous, 5-partite, the segments being more or less linear, entire, with acute apices. In phylloid flowers, a complete division of the calyx tube occurs, resulting in a polysepalous calyx. The five sepals thus produced are sessile (occasionally sub-sessile), lanceolate or ovate-lanceolate, entire, with acute apices; they are rather leaf-like in appearance but are much smaller and possess a simpler configuration than foliage leaves. The venation is characteristic, the 3-7 primary veins being thickened and prominent. The internal structure of the phylloid sepal is similar to


1
2
3
4
5
6
7
8
p
e
9
p
g
10
11
a
b
c
12

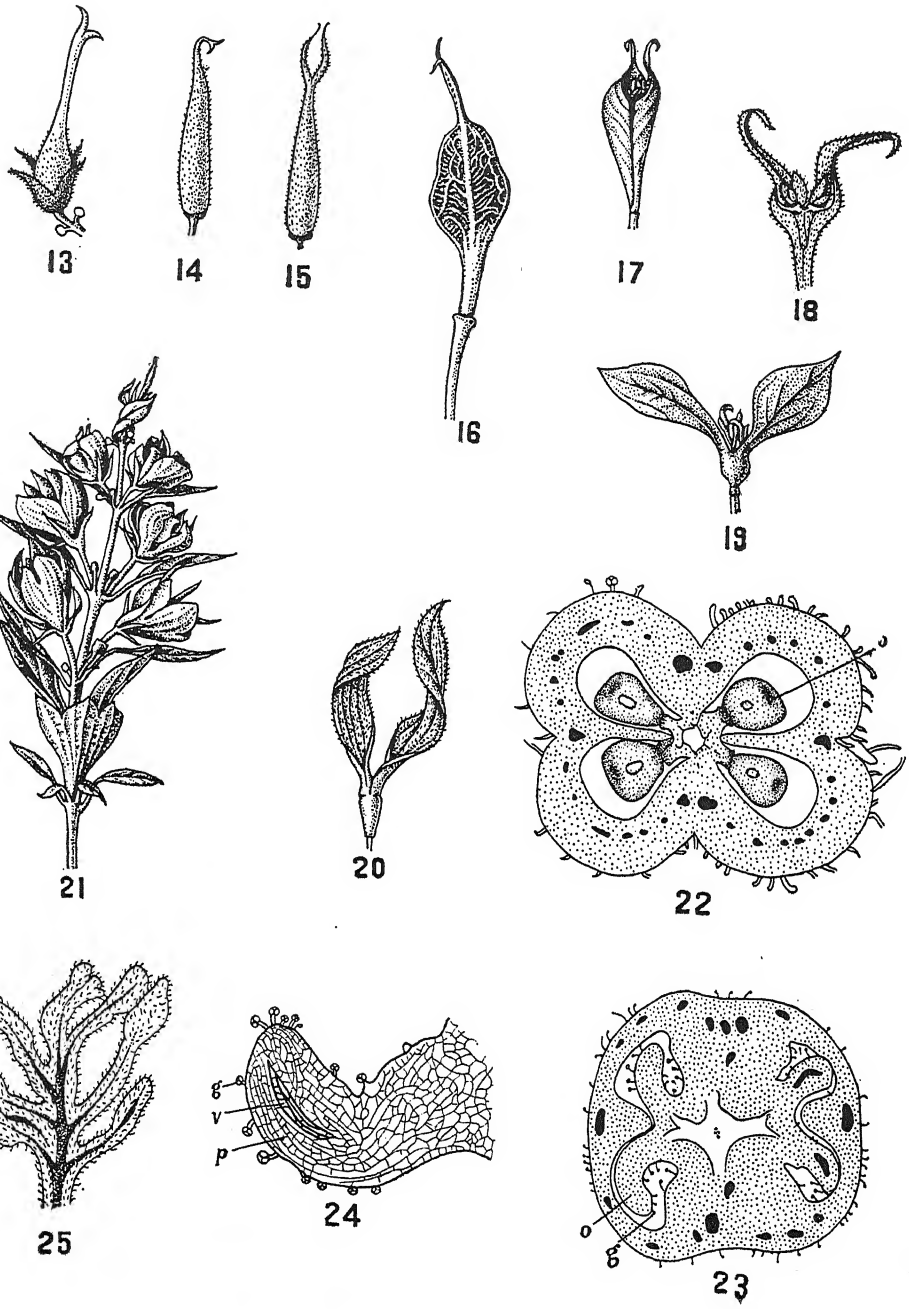

13
14
15
16
17
18
19
21
20
22
25
g
v
p
24
o
g
23

Fig. 13. Normal ovary. (× 2)

„ 14-20. Modifications of ovary in phylloid flowers. Figs. 14, 15, and 17, (× ⅘); 18, 19, and 20 (× 2); 16, (⅔ nat. size).

„ 21. Shoot arising from centre of phylloid flower and bearing "secondary" phylloid flowers. (⅔ nat. size).

„ 22. T. S. normal ovary. o=ovule. (× 18)

„ 23. T. S. abnormal ovary. g=glandular hair, o=outgrowth replacing ovule. (× 18)

„ 24. Outgrowth (o) in Fig. 23 enlarged to show lack of differentiation. g=glandular hair, p=parenchyma, v=vascular strand.

„ 25. Part of placenta from phylloid flower showing abnormal outgrowths produced in place of ovules. (× 7)

that of a foliage leaf but the venation is multicostate instead of being unicostate as in the latter.

Corolla (*Plate XXIV, figs. 1-3, 5*).—The margin of the corolla, or the whole of it, becomes green according to whether the phyllody is partial or complete; the surface is often rough, due to the veins being unusually thickened. Actinomorphic symmetry is acquired and the gamopetalous condition may be changed to a polypetalous one. Phylloid petals are similar in external appearance and internal structure to phylloid sepals except that the apices are rounded.

Andrœcium (*Plate XXIV, figs. 6-12*).—The stamens in the normal flower are four in number, epipetalous and didynamous. In the majority of abnormal flowers a fifth (anterior) stamen is developed and in completely phylloid flowers the stamens are no longer epipetalous (Plate XXIV, fig. 12). Though the stamens retain their normal configuration through all the stages of phyllody they are generally much smaller than normal ones and the anther lobes are usually reflexed. The whole stamen is green in colour.

In normal stamens there is a short connective and four pollen sacs, and the endothecial layer of fibrous banded cells which helps the sacs to dehisce is well developed (Plate XXIV, fig. 8); the pollen grains are dark-brown in colour, about 83—90 μ long and 77—83 μ broad. In phylloid flowers, the region of the connective is much broader, the epidermis shows a few glandular hairs and stomata, and the outer 2-3 cell-layers of both the connective and the pollen-sacs contain chloroplasts. The most distinctive feature, however, is the absence of the endothecial layer: this explains why the anthers do not dehisce. The cavity of the pollen-sac is greatly reduced by the excessive growth of parenchymatous tissue and is either empty or contains a few small (about 39—42 μ long and 33—39 μ broad), light-coloured, non-functional pollen grains in which the exine and intine are not differentiated.

Gynœcium (*Plate XXV, figs. 13-25*).—The chief modifications are (1) an enlargement of the ovary with the gradual loss of the style. The stigma may be transformed into two flattened, greenish structures. The ovary may become flattened and the veins of the carpellary wall are much thickened. (2) The gradual transformation of the carpellary wall to two foliaceous structures. In a few cases the latter are very similar in general configuration and venation to young foliage leaves but in the majority of cases a midrib is lacking and they are more like the phylloid sepals or petals.

The ovules are invariably non-functional in phylloid flowers. In the earliest manifestations of phyllody, a number of outwardly normal capsules may be produced but in these the ovules are replaced by parenchymatous outgrowths bearing glandular hairs. Often, however, small leaf-like structures are produced in place of ovules and in some cases the placenta forms a central axis on which such leaf like outgrowths are borne. In a few extreme cases, a central shoot grows out of

the middle of the flower, bearing phylloid flowers after the manner of the main inflorescence. These "secondary" phylloid flowers may in their turn give off shoots carrying "tertiary" phylloid flowers; such cases, however, occur extremely rarely.

Flowers from 20 affected plants could be classified into the following nine classes:—

1. Sepals leaf-like; corolla gamopetalous and green at margin; ovary enlarged; ovules non-functional.
2. Sepals leaf-like; corolla gamopetalous, green; ovary enlarged; ovules non-functional.
3. Sepals leaf-like; corolla gamopetalous, green; ovary partially leaf-like; ovules leaf-like.
4. Sepals leaf-like; corolla gamopetalous, green; ovary and ovules leaf-like.
5. Sepals leaf-like; corolla polypetalous, green; ovary partially leaf-like; ovules leaf-like.
6. Sepals leaf-like; corolla polypetalous, green; ovary and ovules leaf-like.
7. Sepals leaf-like; corolla polypetalous, green; ovary leaf-like; ovules absent.
8. Sepals leaf-like; corolla gamopetalous, green; ovary leaf-like; ovules absent.
9. All parts except stamens leaf-like, crowded together in no apparent order.

The largest frequencies occurred in classes 6 and 9. None of the 30 Pusa sesamum types was found to be free from phyllody.

Cause

In an attempt to throw some light on the cause of phyllody the influence of the following factors on its incidence was investigated in 1934: (1) time of sowing, (2) heavy manuring, (3) excessive humidity.

(*1*) *Time of sowing.*—To test the influence of this factor five sowings of two early *til* varieties (Pusa Types 3 and 7) were made at intervals of one week, starting from the 2nd August. The experiment was laid out in the form of randomised blocks.

The percentages of plants showing phyllody found in the five batches of sowings were 1·24, 1·58, 0·99, 0·25, 1·09 and 2·33, 1·73, 1·24, 0·34, 0·64, respectively for Type 3 and Type 7. The numbers in the earlier sowings are definitely, though not significantly, higher than those in the later sowings. As it is improbable that early sowing in itself can be responsible for the higher incidence of phyllody, it seems that the conditions obtaining at that time were more

FIG. 26. *a.* normal sesamum plant. *b.* phylloid sesamum plant.

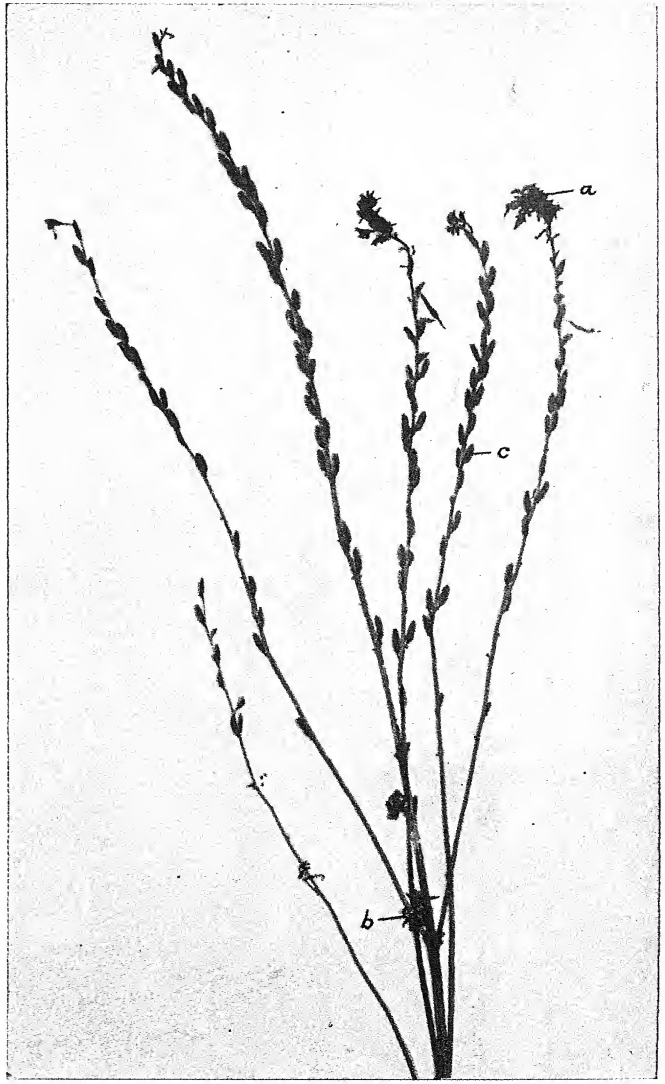


FIG. 27. Sesamum plant which has become phylloid at a very late stage of growth. The tips of the main stem and branches (*a*) and the new shoots from near the base (*b*) are phylloid. The capsules (*c*) were produced by the first-formed normal flowers.

favourable for its occurrence. If a pathogene be concerned then the environmental conditions at the time of the earlier sowings may have been such as to promote its development and spread. It must also be remembered that the earlier the crop is sown, the longer it stands in the field, and, consequently, the longer it is exposed to possible attack.

(2) *Heavy manuring.*—Three treatments were tried, *viz.*, (*a*) Ammophos at 400 lb. per acre, (*b*) farmyard manure at 40 cart-loads per acre, and (*c*) control—no manure. The lay-out was again randomised blocks. The variety used was Type 7. No marked differences in number of phylloid plants per treatment resulted.

(3) *Excessive humidity.*—Sowings of Type 7 were made in three batches (at intervals of one week) in six lysimeters. Half the number of plants in each lysimeter was watered and covered with mats at night in order to promote highly humid conditions, this being done for a week, after the appearance of the first pair of foliage leaves; the other half which served as a control was kept uncovered. In the day time, the mats were removed so as not to interfere with photosynthesis. There were 21 effective plants in each plot (*i.e.*, in each half-lysimeter). Out of the total of 252 plants in all the six lysimeters, however, only one exhibited phyllody.

Inoculation and grafting experiments

Inoculation and grafting were tried to see whether phyllody might be due to a virus. For the inoculation tests, twenty pairs of apparently normal plants were bagged and kept under observation for some time to make sure that they really were healthy. Pieces of phylloid shoots were then taken, washed thoroughly with sterilised water, crushed in a sterilised pestle and mortar, and the extracted juice injected by means of a hypodermic syringe into the stems of twenty plants. The remaining twenty plants which served as controls were injected with the same quantity of sterilised water. All the forty plants remained normal. As only one method of inoculation was attempted, however, it cannot be concluded that juice transmission is impossible.

At the same time as the above, six pairs of plants, each pair consisting of a normal and a phylloid plant, were taken and reciprocally grafted, *i.e.*, the top of the healthy plant (scion) was grafted on to the lower part of the phylloid plant (stock) and *vice versa*. In grafting, the region of the plant adjacent to the place of union was disinfected with absolute alcohol, and the scalpels, etc., employed were sterilised before and after use. Cleft grafting was performed and scion and stock were kept in position by a muslin bandage soaked in molten beeswax. Care was taken to seal the cut ends of the stalks of such leaves as were removed to facilitate grafting. After this the grafted plants were carefully bagged again. Unfortunately strong winds damaged most of the grafts and only four were successful—three grafts wherein the normal plant formed the stock and the phylloid plant the scion, and one of the opposite kind.

After about three weeks the originally normal scion of the last-mentioned grafts was found to be exhibiting symptoms of phyllody, and in the other three grafts, all new branches produced by the originally healthy stock bore phylloid flowers (Plate XXVII). Although the material is too scanty to enable definite conclusions to be drawn, these facts suggest that phyllody in sesamum is in the nature of a systemic disease and is possibly an addition to the growing list of plant viruses. Such an assumption is in consonance with the recorded observations on the incidence of phyllody and may explain, for example, why phyllody should be liable to reveal itself without apparent relation to the environmental conditions prevailing at the time.

Summary

Phylloid *til* plants are characterised by the transformation of all floral parts except the stamens into leaf-like structures. The different modifications encountered are briefly described.

An attempt was made to discover the cause of phyllody and it was found that neither heavy manuring nor the growing of plants under artificially contrived conditions of high humidity sensibly increased the percentage of phylloid plants. It was observed, however, that early sowings contained a larger proportion of them than late sowings.

Hypodermic injection of juice extracted from phylloid plants into the stems of normal plants gave negative results but it was found possible to transmit the phylloid condition by grafting normal scions on phylloid stocks and *vice versa*. This suggests—although the evidence is not conclusive—that phyllody may be caused by a virus.

REFERENCES

Deighton, F. C. (1932). *Ann. Rept. Dept. Agric. Sierra Leone*, 1931, p. 23.

Kashi Ram (1930). *Mem. Dept. Agric. Ind., Bot. Ser.* **18**, 144.

Robertson, H. F. (1928). *Rept. Mycologist, Burma*, 1928, p. 5.

Roy, S. C. (1931). *Agric. & Livestock in India* **1**, 282.

Storey, H. H. (1933). *Fifth Ann. Rept. East African Agric. Res. Stn. Amani*, 1932-33, p. 16.

Wallace, G. B. (1933). *Ann. Rept. Dept. Agric. Tanganyika Territory*, 1932, p. 79.

——————(1934). *ibid* 1933, p. 78.

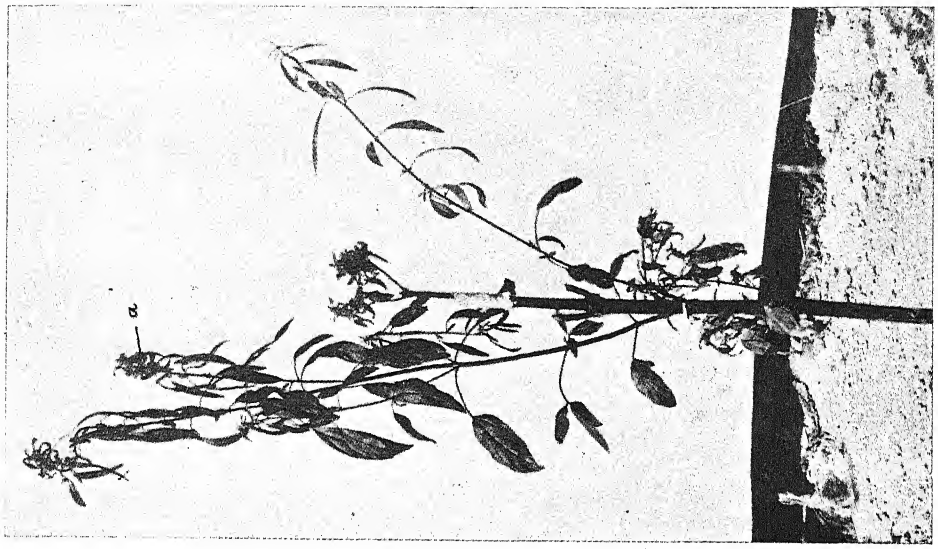


FIG. 29. Phylloid scion grafted on a normal stock. The new growth produced by the latter after grafting (*a*) shows obvious symptoms of phyllody.

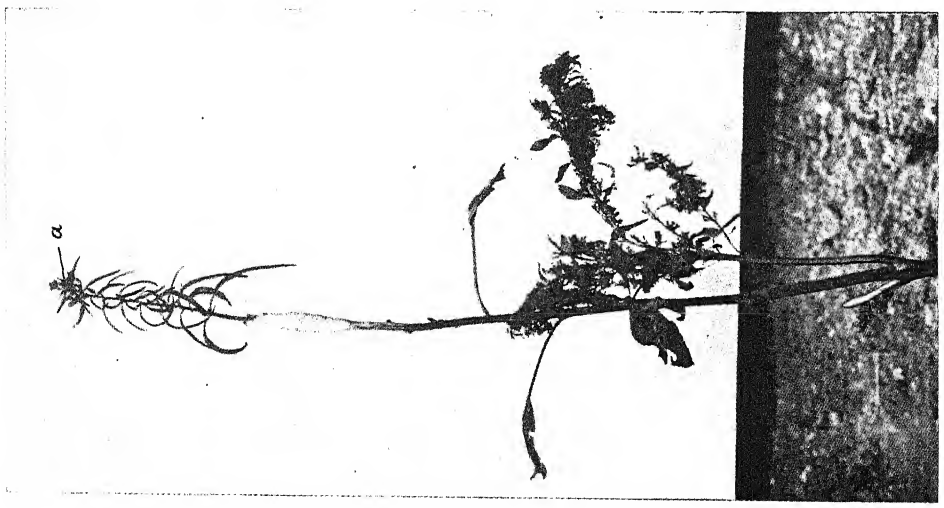


FIG. 28. Normal scion grafted on a phylloid stock. The former (*a*) is just showing symptoms of phyllody.

STUDIES IN THE TECHNIQUE OF FIELD EXPERIMENTS

I. SIZE, SHAPE AND ARRANGEMENT OF PLOTS IN COTTON TRIALS

BY

J. B. HUTCHINSON,

AND

V. G. PANSE,

Institute of Plant Industry, Indore, Central India

(Received for publication on 5th June 1935)

(With one text-figure)

CONTENTS

Foreword

The development at the Institute of Plant Industry of an extensive experimental programme for fundamental research on cotton and for the amelioration of agricultural conditions in States in Central India and Rajputana has made necessary a number of investigations in statistical technique, and has provided opportunities for the study of a number of problems of design and analysis of experiments which are of general interest.

The work in progress falls naturally under two heads, first, the study of methods of increasing the accuracy of varietal trials for plant breeding purposes where rigorous scientific control is possible, and secondly, the development of a satisfactory simplified technique for trials carried out under non-technical control at out-stations or on cultivators' fields.

It is proposed to publish the results of these studies in a series of papers of which this is the first. This paper deals with investigations on the effect of size and shape of plots and blocks on standard error in replicated cotton trials. Further papers will deal with sampling for staple length measurement in cotton, the use of the method of covariance in breeding for disease resistance in cotton, and the effect of discarding margins on the accuracy of comparison in cotton and wheat trials.

J. B. H.

I. Introduction

Variety trials on cotton have been carried out by the Institute in Central India and Rajputana for the past three seasons using Latin Square and randomised block lay-outs. Plot sizes and shapes have been determined largely by convenience and such guidance as could be obtained from the literature on other crops. In 1933 it was decided to lay out a uniformity trial with Malvi mass-selected cotton at Indore in order to acquire more accurate information concerning the optimum conditions of plot size, shape and arrangement for cotton trials. The experiment was laid out at Indore chiefly for convenience of management, and conclusions concerning such matters as the magnitude of the standard error with plots of a certain size and shape are, of course, valid only for the conditions under which it was conducted. It is quite impossible to carry out such trials in all or even a number of the places where experimental work is to be done, but as experimental error has been of the same order in experiments carried out in various parts of Central India and Rajputana as at Indore, it is reasonable to suppose that conclusions reached at Indore concerning the optimum conditions of experimentation will be a fair guide for designing experiments in other areas.

II. Material

The experiment was carried out on a field of reasonably uniform fertility, so far as could be determined from inspection of crops of *jowar* (*Andropogon Sorghum*) and cotton grown in 1930 and 1931. The mean yield per acre of seed cotton from the experimental crop was 345 lbs. as against an average of 400 lbs. per acre given by the local control in ten varietal trials carried out on black soil in Malwa in 1932 and 1933. The field may, therefore, be classed as rather below average in fertility.

One hundred and thirty-eight rows each 206 ft. 8 in. long and 14 in. apart were sown north and south with a two-coultered seed dril (locally called *phadak*) at the end of June when rains began. Seed is dropped into the funnel of the *phadak* by hand, and a measured quantity of seed was sown in each row. The usual preliminary and post-sowing operations in practice at the Institute were carried out. At the time of harvest five rows on each margin of the field and ten feet at each end were discarded to avoid border effect. The central 128 rows were then divided into sections each four rows wide and 4 ft. 8 in. long. There were thus 1280 ultimate units, square in shape and 1/2000 acre in area. Four

pickings were made between the third week of November 1933 and the middle of January 1934. The data in this paper were calculated from the total yield (in grms.) of seed cotton per unit.

The units were combined to give plots 1, 2, 4 and 8 units broad and 1, 2, 4, 5, 8, 10, 20 and 40 units long. (Throughout the paper length will be used for length along the rows, and breadth, for breadth across the rows, irrespective of which dimension is the greater). These plots were grouped together (1) in blocks of four plots in a row (2) in blocks of eight plots in a row and (3) in blocks of eight plots in two rows of four.

By comparisons of different arrangements of units in plots, the effect of plot size and shape on plot error will be studied, and by comparisons of different numbers and arrangements of plots in blocks the relative efficiency of different block arrangements in removing variation due to major soil differences will be investigated.

III. Statistical analysis

In Table I are given percentage standard errors per plot for different plot sizes. The Table is subdivided into three sections to give standard errors per plot for the three types of block separately. In each case variation between blocks has been removed. The effect of this will be discussed below. It is sufficient to note here that the standard error is in general reduced by 50 to 70 per cent by the elimination of block differences (Table IV).

Table I

Standard errors per cent for plots of different sizes and shapes

Breadth in units	Length in units							
	1	2	4	5	8	10	20	40
	A. 4-plot blocks							
1	25·2	18·8	14·4	13·8	11·0	10·0	8·4	6·4
2	19·1	15·3	12·2	11·7	10·2	9·7	8·1	5·5
4	15·4	13·1	10·6	10·5	8·8	8·9	6·9	3·5
8	15·9	14·4	12·3	11·5	9·4	10·7	4·9	3·0
	B. 8-plot blocks in one row							
1	26·2	20·1	15·6	15·2	12·3	11·4	9·5	6·8
2	19·4	15·7	12·6	12·1	10·4	10·1	8·2	5·2
4	18·9	16·7	14·0	13·4	11·1	11·9	7·1	4·3
	C. 8-plot blocks in two rows							
1	25·9	20·1	17·2	14·3	..	17·4	11·9	..
2	20·1	16·3	15·0	11·8	..	16·8	10·8	..
4	16·6	14·0	13·5	10·5	..	16·1	9·5	..
8	16·4	13·9	13·2	10·8	..	15·0	8·3	..

It will be seen that there is a gradual decrease in percentage error per plot as the plot size increases by increase either in length or breadth; but with increase in length reduction in error is more rapid than with increase in breadth. The advantage of increased breadth rapidly diminishes until in the extreme case plots 8 units broad in Table I-A and 4 units broad in Table I-B are actually less advantageous than corresponding plots of half the breadth for all lengths except the two longest. Similarly plots 8 units broad in Table I-C show no advantage over corresponding plots of half the breadth. Table I thus brings out in general the advantage of bigger plots over smaller ones, and of long plots over broad ones. The standard errors per plot in 8-plot blocks are higher for all plot sizes than those in 4-plot blocks. When the 8 plots in a block were arranged in two rows instead of one, no advantage was secured over the single row arrangement and with plots 10 and 20 units long where the two-row arrangement was the less compact, it was actually less advantageous.

Tables II and III are arranged to demonstrate the effects of plot size and plot shape separately.

In Table II plots of different sizes but of the same shape are grouped together. For each shape, increase in plot size has consistently resulted in a decrease in standard error.

TABLE II

Standard errors per cent of plots of different sizes arranged according to shape

Plot shape (Breadth : Length)	Plot size (acre)	Per cent standard error per plot		
		4-plot blocks	8-plot blocks	
			1 row	2 rows
4 : 1	1/500	15·4	18·9	16·6
	1/125	14·4	..	13·9
2 : 1	1/1000	19·1	19·4	20·1
	1/250	13·1	16·7	14·0
	1/62·5	12·3	..	13·2

Plot shape (Breadth : Length)	Plot size (acre)	Per cent standard error per plot		
		4-plot blocks	8-plot blocks	
			1 row	2 rows
1 : 1	1/2000	25·2	26·2	25·9
	1/500	15·3	15·7	16·3
	1/125	10·6	14·0	13·5
	1/31·2	9·4	..	..
1 : 1·25	1/100	10·5	13·4	10·5
	1/25	10·7	..	15·0
1 : 2	1/1000	18·8	20·1	20·1
	1/250	12·2	12·6	15·0
	1/62·5	8·8	11·1	..
1 : 2·5	1/200	11·7	12·1	11·8
	1/50	8·9	11·9	16·1
	1/12·5	4·9	..	8·3
1 : 4	1/500	14·4	15·6	17·2
	1/125	10·2	10·4	..
1 : 5	1/400	13·8	15·2	14·3
	1/100	9·7	10·1	16·8
	1/25	6·9	7·1	9·5
	1/6·25	3·0	..	..
1 : 10	1/200	10·0	11·4	17·4
	1/50	8·1	8·2	10·8
	1/12·5	3·5	4·3	..
1 : 20	1/100	8·4	9·5	11·9
	1/25	5·5	5·2	..

In Table III plots of different shapes but of the same size are grouped together For each plot size, longer plots have in general given lower standard errors than broader plots.

TABLE III

Standard errors per cent of plots of different shapes arranged according to size

Plot size (acre)	Plot shape (Breadth : Length)	Per cent standard error per plot		
		4-plot blocks	8-plot blocks	
			1 row	2 rows
1/2000	1 : 1	25·2	26·2	25·9
1/1000	2 : 1	19·1	19·4	20·1
	1 : 2	18·8	20·1	20·1
1/500	4 : 1	15·4	18·9	16·6
	1 : 1	15·3	15·7	16·3
	1 : 4	14·4	15·6	17·2
1/400	1 : 5	13·8	15·2	14·3
1/250	8 : 1	15·9	..	16·4
	2 : 1	13·1	16·7	14·0
	1 : 2	12·2	12·6	15·0
	1 : 8	11·0	12·3	..
1/200	1 : 2·5	11·7	12·1	11·8
	1 : 10	10·0	11·4	17·4
1/125	4 : 1	14·4	..	13·9
	1 : 1	10·6	14·0	13·5
	1 : 4	10·2	10·4	..
1/100	1 : 1·25	10·5	13·4	10·5
	1 : 5	9·7	10·1	16·8
	1 : 20	8·4	9·5	11·9
1/62·5	2 : 1	12·3	..	13·2
	1 : 2	8·8	11·1	..
1/50	1·6 : 1	11·5	..	10·8
	1 : 2·5	8·9	11·9	16·1
	1 : 10	8·1	8·2	10·8
	1 : 40	6·4	6·8	..
1/31·2	1 : 1	9·4	..	..
1/25	1 : 1·25	10·7	..	15·0
	1 : 5	6·9	7·1	9·5
	1 : 20	5·5	5·2	..
1/12·5	1 : 2·5	4·9	..	8·3
	1 : 10	3·5	4·3	..
1/6·25	1 : 5	3·0	..	..

Table III also brings out the relative importance of size and shape of plots in reducing error. With the smaller plot sizes, the effect of size is predominant, so that larger plots are more efficient than smaller ones, irrespective of shape. With larger plot sizes, shape is considerably more important, so that broad plots are often less efficient than long plots of a smaller size. Plot shape is of most importance within the range of plot sizes usually employed in field experiments, and it is clear that the best use will be made of land if plots are made as long as is consistent with border row requirements and convenience of field arrangement.

It is a striking observation that in the present study, for any given plot shape, a considerably lower error was obtained when the longer dimension of the plot was along the rows than when it was across the rows. The most obvious explanation is that there may be a fertility gradient along the direction of sowing. A fertility contour map of the field was compiled from the yield data and is given in Fig. 1. Inspection shows that in general, and particularly on the western side of the field, a long plot running north and south (along the rows) will cross more of the fertility contour lines, and will therefore average out fertility differences better, than a plot of the same shape with its long dimension east and west. It was decided to take a square 20 units × 20 units from the northern half of the field (outlined in dotted lines in Fig. 1) and to correct the yields of the 400 units in it, for the regression of yield on position. The two regression coefficients were :—

N-S $b_1 = +2{\cdot}09$, $\sigma\ (b_1) = 0{\cdot}48$, $P = 0{\cdot}01$

E-W $b_2 = -0{\cdot}61$, $\sigma\ (b_2) = 0{\cdot}30$, $P = 0{\cdot}06$.

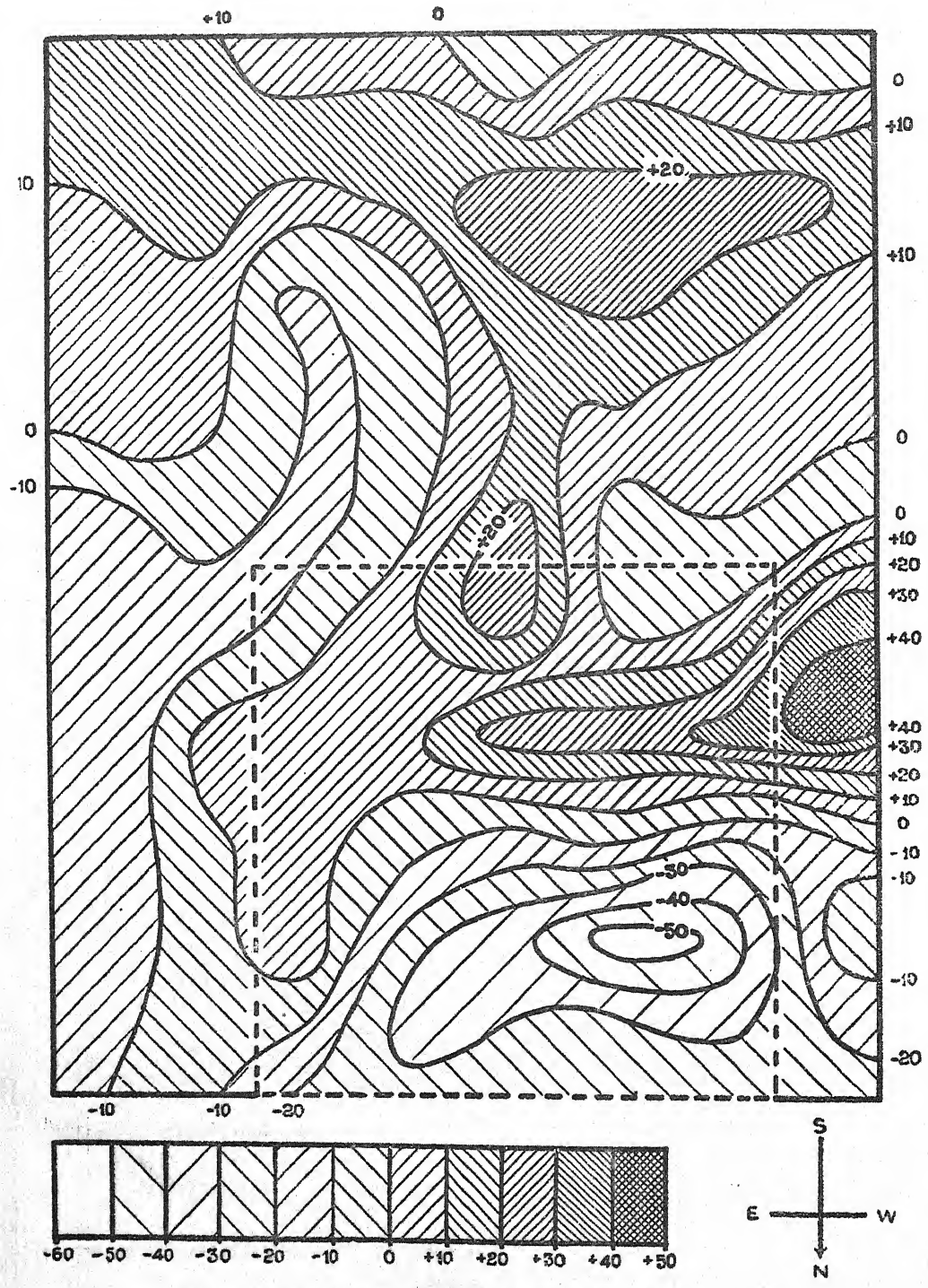


Fig. 1. Fertility contour map from data of cotton uniformity trial.

Plots of 20 units each were compiled in two ways, (1) with a breadth : length ratio of 1 : 20, and (2) with a breadth to length ratio of 20 : 1. Standard errors per cent per plot were calculated from the corrected plot yields and gave—

Along the rows (1 : 20) $\sigma = 10 \cdot 9$ per cent
Across the rows (20 : 1) $\sigma = 17 \cdot 6$ per cent
P (diff.) $= 0 \cdot 05$

Elimination of the fertility gradient, therefore, did not destroy the advantage of long plots over broad ones. Consideration of the method of sowing gives a reasonable explanation of the advantage, in that a measured amount of seed was sown in each row, and no doubt the individual dropping the seed into the *phadak* would tend to compensate any excesses in one section of the row by a deficiency in the next, and *vice versa*. This would result in greater variation between short lengths of the same row than between equal lengths of neighbouring rows. The longer the section of a row included in the plot, therefore, the less will be the variability in amount of seed sown per plot. In varietal trials an equal amount of seed is normally sown in each plot, and differences in seed rate will, therefore, not contribute to error. In agronomic trials, however, where seed is sown uniformly over a number of treatments, the arrangement of treatments to include long lengths of rows or even the whole rows may contribute materially to the reduction of error. A particular case is that in which each main plot in a confounded [Yates, 1933] experiment is divided in halves. Such divisions should wherever possible be along the rows and not across them.

In Table IV are given percentages of the total sums of squares removed by blocks of different sizes and shapes.

TABLE IV

Percentage variation removed by blocks of different sizes and shapes

4-plot blocks			8-plot blocks				
				One row		Two rows	
Block size (acre)	Block shape (Breadth : Length)	Percentage sum of squares	Block size (acre)	Block shape (Breadth : Length)	Percentage sum of squares	Block shape (Breadth : Length)	Percentage sum of squares
1/500 .	4 : 1	53·4	1/250	8 : 1	40·1	2 : 1	42·3
1/250 .	8 : 1	56·1	1/125	16 : 1	52·9	4 : 1	49·3
	2 : 1	61·9		4 : 1	49·1	1 : 1	49·3

4-plot blocks			8-plot blocks				
				One row		Two rows	
Block size (acre)	Block shape (Breadth : Length)	Percentage sum of squares	Block size (acre)	Block shape (Breadth : Length)	Percentage sum of squares	Block shape (Breadth : Length)	Percentage sum of squares
1/125	16 : 1	67·6	1/62·5	32 : 1	42·6	8 : 1	55·7
	4 : 1	66·2		8 : 1	58·6	2 : 1	55·4
	1 : 1	68·8		2 : 1	57·4	1 : 2	48·5
1/100	1 : 1·25	68·7	1/50	1·6 : 1	56·0	1 : 2·5	60·7
1/62·5	32 : 1	55·1	1/31·25	..	..	16 : 1	44·4
	8 : 1	70·3		16 : 1	43·4	4 : 1	60·1
	2 : 1	72·2		4 : 1	65·3	1 : 1	50·5
	1 : 2	72·7		1 : 1	60·2	..	..
1/50	1·6 : 1	71·3	1/25	3·2 : 1	64·3	1 : 1·25	66·3
	1 : 2·5	78·8		1 : 1·25	67·6	1 : 5	24·4
1/31·25	16 : 1	54·7	1/15·6	..	..	8 : 1	50·8
	4 : 1	75·4		8 : 1	50·6	2 : 1	53·7
	1 : 1	71·9		2 : 1	66·2	..	..
1/25	3·2 : 1	73·6	1/12·5	6·4 : 1	49·9	1·6 : 1	69·5
	1 : 1·25	77·3		1·6 : 1	70·7	1 : 2·5	19·4
	1 : 5	61·9		1 : 2·5	43·1	1 : 10	11·5
1/15·6	8 : 1	60·6	1/7·8	..	..	4 : 1	46·9
	2 : 1	76·2		4 : 1	55·5	..	..
1/12·5	6·4 : 1	61·2	1/6·25	..	..	3·2 : 1	59·7
	1·6 : 1	78·6		3·2 : 1	55·1	1 : 1·25	18·2
	1 : 2·5	55·1		1 : 1·25	46·4	1 : 5	6·2
	1 : 10	34·1		1 : 5	14·4	..	..
1/7·8	4 : 1	67·0	1/3·12	..	..	1·6 : 1	17·1
1/6·25	3·2 : 1	64·2		1·6 : 1	49·0	1 : 2·5	7·6
	1 : 1·25	58·6		1 : 2·5	21·9	..	..
	1 : 5	22·7					
1/13·12	2·6 : 1	70·4					

Four-plot blocks remove a larger proportion of the variation than eight-plot blocks of the same size and shape. In both four-plot and eight-plot blocks, blocks less than 1/100 acre remove a smaller proportion of the variation than blocks of 1/100 to 1/25 acre. Blocks of the latter range of sizes remove about 70 per cent of the variation in four-plot blocks and about 60 per cent in eight-plot blocks. With larger blocks a smaller proportion of the variation is removed and the proportion removed is largely affected by the shape of the block. With block sizes of 1/125 acre or less, block shape has little effect. As the block size increases above this point the efficiency of both long, narrow blocks and short, broad blocks rapidly decreases. With blocks of 1/25 acre or larger, the efficiency of all blocks with breadth greater than six times the length, or less than half the length, is very low.

Advantage was taken of the data collected to compare the efficiency of randomised block and Latin Square lay-outs. Two arrangements were taken, 64 plots arranged 8×8, each plot 1/100 acre in area, and $1 : 1 \cdot 25$ in shape, and 16 plots arranged 4×4, each plot 1/25 acre in area and $1 : 1 \cdot 25$ in shape. Analyses of variance are given below (Table V).

TABLE V

Analyses of variance of 8×8 and 4×4 Latin Squares

	Degrees of freedom	Sum of squares	Mean square
	8×8		
Rows	7	2,453,373	350,482
Columns	7	250,501	35,786
Residual	49	2,214,014	45,184
	4×4		
Rows	3	9,584,007	3,194,669
Columns	3	422,606	140,869
Residual	9	4,927,459	547,495

The mean square appropriate to error in the Latin Square arrangement is the residual mean square in each case. That appropriate to the randomised block arrangement can be obtained from Table V in two ways, by pooling with the residual sum of squares either the column or the row sum of squares and dividing by the appropriate number of degrees of freedom. It is immediately clear that the result obtained will be very different according to whether rows or columns are pooled with residuals. Below are given standard errors per cent per plot obtained as above for Latin Square and randomised block arrangements.

		8 × 8	*4 × 4*
Latin Square		13·6	11·8
Randomised blocks	Row-wise	13·4	10·7
	Column-wise	18·4	17·6

(Row-wise results from pooling columns with residual, and column-wise by pooling rows with residual.)

Clearly, if there is sufficient knowledge to design blocks in the most advantageous manner, randomised blocks can give as efficient a lay-out as a Latin Square. The required information is provided by the discussion of the influence of block shape on proportion of variation removed by blocks (*see* above). The percentages of the variation removed by blocks of the shapes constructed row-wise and column-wise are abstracted from Table IV and given below :—

Blocks	Block shape	Percentage variation removed
8-plot blocks, 1/12·5 acre		
Row-wise	6·4 : 1	49·9
Column-wise	1 : 10	11·5
4-plot blocks, 1/6·25 acre		
Row-wise	3·2 : 1	64·2
Column-wise	1 : 5	22·7

The failure of the Latin Square arrangement to give a lower error than an efficient randomised block arrangement may be ascribed to the fact that columns in the Latin Square represent blocks of a very inefficient shape. In the Latin Square arrangement, therefore, the number of degrees of freedom available for the estimation of error is reduced without any corresponding advantage. Eden and Fisher [1929], and Lord [1931] have previously discussed the failure of a Latin Square to give a lower error than randomised blocks.

IV. Discussion and conclusions

The drawing of conclusions concerning the conditions for efficiency in experimental lay-outs will be facilitated by a consideration of the nature of the statistical manipulations which have been carried out. A constant total variance—the variance between the 1/2000 acre units—has in every case been divided into three parts :—

(1) Variance included within plots.

(2) Variance between plots within blocks.

(3) Variance between blocks.

In experimental data item (1) does not appear and item (3) is eliminated in the statistical analysis. Item (2) remains as an estimate of plot error. The primary statistical consideration in experiment design is, therefore, to include as large a proportion as possible of the uncontrolled variability in items (1) and (3), and thereby to minimise item (2). Two important conclusions on this point have already been indicated, first, in order to maximise item (1), plots should be long and narrow, and secondly in order to maximise item (3), blocks should be approximately square.

Considerations of plot and block size are closely associated with matters of agricultural convenience, amount of land or seed available, and probable efficiency of supervision. It is therefore, desirable to present the conclusions in a manner which will enable the investigator to choose for himself the most efficient design consistent with the facilities available, rather than to attempt to define an optimum lay-out on statistical ground alone. With this aim in view the number of replications and the amount of land required to give a standard error of two per cent of the mean have been calculated for plots of different sizes and shapes, arranged in 4-plot and 8-plot blocks (Table VI). In the 8-plot blocks only the more efficient of the two arrangements of plots considered above has been included.

TABLE VI

Number of replications and area of land required to give a standard error of two per cent of the mean with different plot sizes and shapes

Plot size (acres)	Plot shape	4-plot blocks		8-plot blocks		
		No. of replications	Total area (acres)	Arrangement of plots	No. of replications	Total area (acres)
1/2000 . .	1 : 1	159	0·32	4 × 2	168	0·67
1/1000 . .	2 : 1	91	0·36	8 × 1	94	0·75
	1 : 2	88	0·35	8 × 1	101	0·81
1/500 . .	4 : 1	59	0·47	4 × 2	69	1·10
	1 : 1	58	0·46	8 × 1	62	0·99
	1 : 4	52	0·42	8 × 1	61	0·98
1/400 . .	1 : 5	48	0·48	4 × 2	51	1·02
1/250 . .	8 : 1	63	1·01	4 × 2	67	2·14
	2 : 1	43	0·69	4 × 2	49	1·57
	1 : 2	37	0·59	8 × 1	40	1·28
	1 : 8	30	0·48	8 × 1	38	1·21
1/200 . .	1 : 2.5	34	0·68	4 × 2	35	1·40
	1 : 10	25	0·50	8 × 1	32	1·28
1/125 . .	4 : 1	52	1·66	4 × 2	49	3·14
	1 : 1	28	0·89	4 × 2	45	2·88
	1 : 4	26	0·83	8 × 1	27	1·73
1/100 . .	1 : 1·25	27	1·04	4 × 2	27	2·16
	1 : 5	23	0·92	8 × 1	25	2·00
	1 : 20	18	0·72	8 × 1	22	1·76
1/62·5 . .	2 : 1	38	2·44	4 × 2	43	5·51
	1 : 2	19	1·22	8 × 1	31	3·97
1/50 . .	1.6 : 1	33	2·64	4 × 2	29	4·64
	1 : 2·5	20	1·60	8 × 1	35	5·60
	1 : 10	16	1·28	8 × 1	17	2·72
	1 : 40	10	0·82	8 × 1	11	1·76
1/31·2 . .	1 : 1	22	2·82	..	..	..
1/25 . .	1 : 1·25	29	4·64	4 × 2	56	7·95
	1 : 5	12	1·92	8 × 1	13	4·17
	1 : 20	7	1·12	8 × 1	7	2·24
1/12·5 . .	1 : 2·5	6	1·92	4 × 2	17	10·90
	1 : 10	3	0·96	8 × 1	5	3·21
1/6·25 . .	1 : 5	2	1·28	..	..	..

A standard error of two per cent of the mean was taken as a standard, a an investigator may reasonably demand experimental methods accurate enough to enable him to rely upon differences of the order of five or six per cent of the mean as significant. If differences of less than 11 per cent can safely be ignored, a standard error of 4 per cent will be sufficiently accurate, and the number of replications and the amount of land required will be one quarter of those given in Table VI.

The reduction in plot error with increasing plot size has already been pointed out. Table VI reflects this in the reduction in the number of replications required to give a 2 per cent error as plot size is increased. The reduction in error is not proportional to the increase in plot size, however, and consequently the amount of land required to give a two per cent error steadily increases as the plot size increases.

The effect of agricultural convenience, limitation of land or seed, and efficiency of supervision may now be considered.

Limited seed is available in the first stages of multiplication of selected strains in plant breeding work. Such limitation is usually confined to stages of work which are normally carried out on experiment stations where adequate supervision is available, and the expenditure of extra time and labour is of less importance than the acquisition, at an early stage, of reliable information concerning the potentialities of the strains under test. Experiments should, therefore, be laid out with small plots and many replications.

For the large majority of experiments carried out on experiment stations, plot size and number of replications can be varied within wide limits. In making a decision on design the following points of convenience should be borne in mind :

(1) Plots should be large enough to allow of the standard agricultural methods of the district being used, in order that the results obtained shall be strictly applicable to local conditions.

(2) Where land is limited, *e.g.*, where experiments are carried out on small areas irrigated from wells, smaller plots and more replications may be required.

(3) With very small plots the area occupied by marginal rows will be out of proportion to the whole. The full efficiency of small plots in reducing the area of land required cannot be obtained unless margins can safely be dispensed with.

The design of experiments for out-stations requires special study. It may be stated here, however, that where skilled supervision is lacking or intermittent, simplicity in design is essential. For this, plots should be large and replications comparatively few. Thus the number of separate plot products to be harvested and weighed is minimised, and the risk of having to weigh small lots on crude scales is avoided. Margins and guard rows should form as small a proportion of the whole as possible.

In Institute experiments it has been usual to employ plots of 1/100 acre with a breadth/length ratio of 1 : 2. With six replications a standard error of 4 per cent of the mean would be expected. In actual fact standard errors have ranged from 5 to 10 per cent of the mean, depending on efficiency of supervision and facilities for accurate execution. In practice, therefore, it may be expected that rather more replications, and rather larger areas will be required for a given standard error than are indicated in Table VI, since there are likely to be sources of error in a multiple plot experiment which do not exist in a uniform crop not subdivided until harvest time. Considerable improvement in accuracy may confidently be expected if plot size is increased from 1/100 acre to 1/50 acre and the plots are made longer in proportion to their breadth.

V. Summary

1. The results of a uniformity trial with Malvi cotton at Indore are reported.

2. Standard error per cent per plot decreased steadily with increasing plot size.

3. For any given plot size standard error per cent per plot decreased steadily as the length of the plot (along the rows) was increased.

4. The advantage of long, narrow plots laid out along the rows over plots of the same size and shape laid out across the rows is shown to be independent of fertility gradient, and it is suggested that it is associated with the method of sowing.

5. It is shown that approximately square blocks eliminate more of the soil heterogeneity than rectangular ones. With blocks of 1/25 acre or larger, shape is the most important factor in determining efficiency.

6. A Latin Square lay-out has no advantage over an efficiently designed randomised block lay-out with the same number of plots.

7. Optimum lay-outs for different types of experimentations are discussed and a table is given to facilitate determination of the amount of land and number of replications required for a given accuracy with different plot sizes.

VI. Acknowledgments

Our thanks are due to Mr. Y. D. Wad, under whose charge the experiment was laid down, before the senior author's arrival, and to whose interest and criticism we have been indebted throughout.

A large part of the laborious computation involved was undertaken by Mr. S. A. Khargonekar, whose assistance we take this opportunity of acknowledging.

References

Eden, T. and Fisher, R. A. (1929). *J. Agric. Sci.* **19**, 201.

Lord, L. (1931). *J. Agric. Sci.* **21**, 178.

Yates, F. (1933). *J. Agric. Sci.* **23**, 108.

INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET

VII. LIGULE AND AURICLE

BY

G. N. RANGASWAMI AYYANGAR, B.A., I.A.S.,

Millets Specialist, Agricultural Research Institute,

V. PANDURANGA RAO, M.A.,

AND

A. KUNHIKORAN NAMBIAR, B.Sc. Ag.,

Assistants, Millets Breeding Station, Coimbatore

(Received for publication on 3rd April 1935)

(With Plate XXVIII)

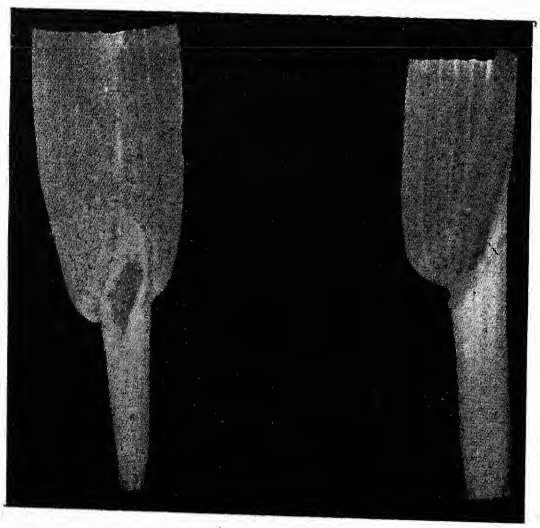
In sorghum, as in all grasses, there is a well-marked zone at the junction of the leaf sheath and the leaf blade. This zone has two parts, (1) a narrow ligule which is a prolongation of the distal inner margin of the leaf sheath, and (2) a triangular, light coloured membranous tissue, which is usually flat, in sorghum, and may vary in the width of its base. In extreme cases as in *Sorghum caudatum* Stapf, the length of the base may be so great as to throw the outline into a curve of a broad sweep giving it an auricular disposition in which the lobes meet and encircle the stem. These structures and their variations are related both to leaf size and leaf disposition and have varietal peculiarities. It will be obvious that the specialised zone at the junction of the leaf blade and leaf sheath plays an important role in the development of the leaf blade by making it possible for the latter to widen out and link up with the clasping leaf sheaths.

A condition in which both the ligule and the junction are absent, though uncommon, has been recorded in the wild grasses as well as in the cultivated cereals. Emerson [1912] first noted this condition in maize and recorded that it behaved as a simple recessive to the presence of ligule and auricle. Vavilov and Bukinich [1929] have noted it as a simple recessive condition in wheat. Brink [1933] finds that two factors are responsible for the ligulate and auriculate condition the absence of either or both of which would give an e-ligulate and non-auriculate plant.

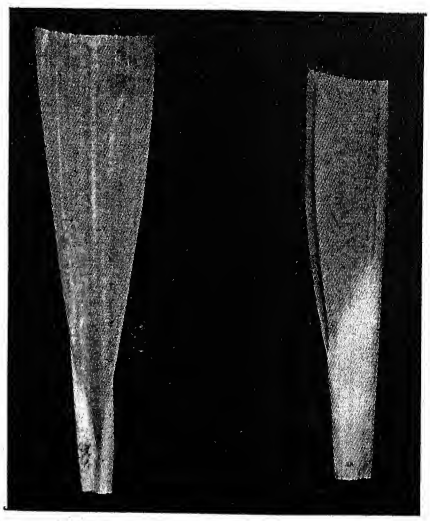
At the Millets Breeding Station, two instances have been met with of this e-ligulate and non-auriculate condition, one in Sudan grass and the other in Broom corn, both being relatively primitive types of sorghum. These forms have probably arisen as mutants from normal ligulate populations and being peculiar and recessive were easily picked out and perpetuated. Pure lines of these e-ligulate and non-auriculate plants were grown at Coimbatore and in both of them some natural crosses occurred possessing both ligule and auricle. Six of these were taken for study and the seedlings raised from them showed clear segregations for the ligulate and auriculate condition and its absence.

NX No.	F_2 seedlings	
	Ligulate and auriculate	E-ligulate and non-auriculate
Sudan grass		
A. S. 3695	320	102
Broom corn—		
A. S. 3696	545	174
„ 3697	322	108
„ 3698	266	79
„ 3699	243	78
„ 3700	236	71
Total (observed)	1,932	612
Total (calculated)	1,908	636

$\chi^2 = 1 \cdot 21$, $P > \cdot 1$.



Ligulate and auriculate



E ligulate and non-auriculate

It will be seen that this e-ligulate and non-auriculate condition is due to the absence of a single factor that behaves as a simple recessive to its presence. Whether more than one factor is involved in this phenomenon only wider experiences can show. It will be seen from the illustration (Plate XXVIII) that in the absence of the auricular adaptation, the seedlings have their leaves erect and close in marked contrast to the free and wide disposition of the normal seedlings. The adult plants also showed this difference. This appearance of a primitive character, probably as a result of a new environment, is interesting as throwing light on the evolution of the ligule and auricle. Moreover in maize a linkage has been established between the factor for ligule and the **Rg** factor for raggedness in the blade [Brink and Senn, 1931]. The normal ligulate and auriculate character has been designated **Lg** to bring it into line with the nomenclature used for maize.

Summary

An e-ligulate and non-auriculate condition has been met with in the leaves of sorghum. This character has been designated **lg** , the normal ligulate and auriculate condition being **Lg**. **Lg** is a simple dominant to **lg**.

REFERENCES

Brink, R. A. (1933). *J. Hered.* **24**, 325.

Brink, R. A., and Senn, P. H. (1931). *J. Hered.* **22**, 155.

Emerson, R. A. (1912). *Neb. Agric. Exp. Stn. Rep.* **25**, 81.

Vavilov, N. I. and Bukinich, D. D. (1929). *Supp. 33 to Bull. Appl. Bot. Gen. and Plt. Breed. Leningrad,* 1929.

NOTES.

ROUND-TABLE DISCUSSION ON NUTRITION AT THE NEXT CONGRESS OF THE FAR EASTERN ASSOCIATION OF TROPICAL MEDICINE

We have received the following circular letter from the local Secretary of the Far Eastern Association of Tropical Medicine :—

At the next Congress of the Far Eastern Association of Tropical Medicine, it is proposed to hold a round-table discussion on nutrition, and we have been asked by the Council to make preparation for it.

Papers are invited upon Nutrition from the widest point of view and we should be glad if you will be so good as to ask trained observers who are working on any aspect of the subject in your country, whether they will kindly contribute to the discussion by reading a paper on their work under any of the sub-headings below.

If suitable support is forthcoming, it may be possible to combine the papers received and the discussions in a volume, which would constitute an up-to-date account of Nutrition as concerns the East.

It is hoped that some indication of the support which may be expected from your country may be received during 1935 though it will not be necessary for titles of papers to be sent in until a later date which will be notified in due course. Such co-operation will enable the Council to know how much time should be allotted for the discussion.

It has been proposed to divide papers under three headings as follows :—

I. Economics.—To include such aspects as agriculture in relation to human nutrition, *e.g.*, improvement of yield and quality of food crops ; horticulture ; fruit-growing ; stock-raising ; dairy problems ; institutional feeding ; food surveys ; storage ; cooking, etc.

II. Chemical and physiological.—To include food analyses in the widest sense ; vitamin, mineral, fat, protein studies, etc.; metabolism, basal metabolism, energy requirements, specific dynamic action.

III. Clinical.—Studies of disease in relation to food and diet, the feeding of infants during the first year with special reference to development (height and weight) ; children's diseases in relation to food ; nutritional œdema, atypical beriberi ; the course of infectious diseases

under the influence of food ; liver cirrhosis ; anæmias ; skin diseases in relation to food and vitamins ; ulcers of the leg ; leprosy in relation to food ; constitutional diseases ; diabetes ; obesity ; gallstones ; gastric ulcer, etc.; clinical value of certain foods, etc.

It should be understood that the above provisional programme is intended to be as wide as possible, and that additional suggestions from those able to make them will be welcomed. It is hoped that the subject of nutrition will receive emphasis from the general and normal point of view as well as from the point of view of disease.

IMPORTATION OF 'MEXICAN JUMPING BEANS' INTO BURMA

In exercise of the powers conferred by sub-section (i) of section 3 of the Destructive Insects and Pests Act, 1914 (II of 1914), the Governor General in Council has been pleased to direct that the following further amendment shall be made in the Order published with the Notification of the Government of India in the late Department of Revenue and Agriculture, No. 580-240, dated the 22nd June 1922, namely :—

Paragraph 8 of the said Order shall be re-numbered "8(a)" and after the paragraph as so re-numbered the following sub-paragraph shall be inserted namely :—

"(b) The importation of 'Mexican Jumping Beans', (*Sebastiania palmeri* of the family Euphorbiaceae is prohibited absolutely."

GIPD—61 IC of AR—23-8-35—800.

ORIGINAL ARTICLES

STUDIES IN THE TECHNIQUE OF FIELD EXPERIMENTS

II. SAMPLING FOR STAPLE-LENGTH DETERMINATION IN COTTON TRIALS

With a Note on the Standard Error of Estimates of Ginning Percentage

BY

J. B. HUTCHINSON

AND

V. G. PANSE,

Institute of Plant Industry, Indore, Central India

(Received for publication on 19th June 1935)

(With one text-figure)

Staple-length in cotton is usually estimated for plant-breeding purposes by measuring the maximum halo-length on a number of seeds on which the lint has been combed out with a weaver's comb. At Indore the maximum halo-length is measured on one side of each of 10 combed seeds. Measurements are made with Bailey's [1930] staple-length measurer.

It was known that the variance of the mean of ten halo-lengths is low, and it was hoped that it might be possible to reduce the number of measurements without seriously reducing the accuracy of the mean. No published information appeared to be available concerning variation in staple-length from plot to plot of the same variety. If variation between plots of a variety were of the same order as variation within plots, a satisfactory estimate of halo-length, together with a valid standard error, could be made from a single bulk sample of the produce of a variety, thereby saving the labour and storage accommodation involved in keeping separate samples from each plot.

Halo-lengths were measured on samples obtained from two randomised block experiments laid out on *barani* (unirrigated) land at Dhar and Limbodi (near Indore) in 1933. Five Indore selections and the local cotton (all Indian varieties) were tested in six blocks in each experiment, giving 36 plots per experiment. The soil at Limbodi was much less fertile than at Dhar and the yield of the Limbodi trial was further depressed by very late sowing.

Mean yields per acre were :—

Dhar	424 lbs.
Limbodi	169 lbs.

Only one picking was possible at Limbodi and the whole of the produce was available for lint-length sampling. At Dhar three pickings were taken, and the whole produce of each plot was thoroughly mixed and a sample drawn for staple-length determination.

Measurements were made on one side of each of ten combed seeds per sample, making 360 measurements per experiment.

Statistical analyses on a single measurement basis are given in Table I.

TABLE I

Analysis of variance of halo-length measurements

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$
Dhar—				
Blocks . .	5	20	4·00	0·69
Varieties . .	5	486	97·20	2·29
Between plots .	25	334	13·36	1·30
Within plots .	324	407	1·26	0·12
Limbodi—				
Blocks . .	5	50	10·00	1·15
Varieties . .	5	87	17·40	1·43
Between plots .	25	309	12·36	1·26
Within plots .	324	361	1·11	0·05

		Dhar	Limbodi
Between plots v. within plots	$z =$	1·18	1·21
$n_1 = 25$, and $n_2 = 324$			
$P = 0·01$ when z is about 0·3			
Blocks v. between plots	$z =$	0·61	0·11
$n_1 = 25$, and $n_2 = 5$			
$P = 0·05$ when $z = 0·76$			
Varieties v. between plots	$z =$	0·99	0·17
$n_1 = 5$, and $n_2 = 25$			
$P = 0·05$ when $z = 0·48$			
$P = 0·01$ when $z = 0·67$			

Significant differences between varieties exist in the Dhar data only.

In neither experiment was the lint-length variation between blocks as great as the variation between plots within blocks. Arrangement of plots in blocks, therefore, conferred no advantage in the comparison of halo-lengths. In both these experiments the variation in yield between blocks was very much larger than that between plots within blocks, showing that the causes of uncontrolled variation in lint-length are different from those in yield.

The mean square between plots represents the error mean square appropriate for testing the significance of varietal differences, while the mean square within plots represents the sampling error. In both experiments the error mean square was about ten times as large as the sampling error. Plot-to-plot differences are, therefore, responsible for the major portion of the error of halo-length determinations, and such determinations should, wherever possible, be made on each plot separately, and not on bulk samples. The standard error determined from the variation between measurements on seeds drawn from a bulk sample will therefore be a serious under-estimate. Such an estimate of the standard error was obtained by treating the whole of the observations in each of the two experiments under review as a single set of measurements for each variety. The two estimates of the standard error of the mean are compared below :—

	Treated as bulk sample	Plot-wise (From Table I)
Dhar	0·19	0·47
Limbodi	0·18	0·45

Table I shows quite clearly that the accuracy of comparisons of lint-length depends almost entirely on the number of plots available, and only to a very small extent on the number of measurements per plot.

Let K be the variance of the mean of N plots with n measurements per plot. Then, if p be the variance between plots due to soil differences, and m be the variance between single measurements within a plot,

$$K = \frac{p + \frac{m}{n}}{N}$$

[Immer, 1932]

It is now possible to make a direct study of the effect of changes in number of measurements per plot or of number of replications on the accuracy of comparison

of staple-length measurements. In the experiments under discussion the following values were obtained :—

	Dhar	Limbodi
$K = \frac{\text{Mean square (between plots)}}{N\,n}$	0·223	0·206
m = Mean square (within plots) . . .	1·26	1·11
n	10	10
N	6	6
$p = KN - \frac{m}{n}$	1·210	1·125

Changes in the number of measurements per plot (n) will only influence K by changing the value of $\frac{m}{n}$, whereas changes in N affect the value of K directly. Since m is approximately equal to p in the present instance where $n = 10$, $\frac{m}{n}$ only contributes about 10 per cent of the value of K, and number of replications is the predominant factor in determining the value of K. Improvement in accuracy of comparison of staple-length measurements, therefore, can be gained rapidly by increasing the number of replications, but only very slowly and to a very limited extent by increasing the number of measurements per plot.

The number of replications in an experiment is primarily determined by considerations of agricultural convenience, and the size of the error permissible in yield comparisons. It is not usually possible to make N greater than six or eight. The only question for consideration is the extent to which the labour of staple-length determination can be reduced by reducing the number of measurements per plot.

In Fig. I the effect of varying the number of measurements per plot on the variance of the mean of six plots is represented graphically. Calculations are based on the Dhar data.

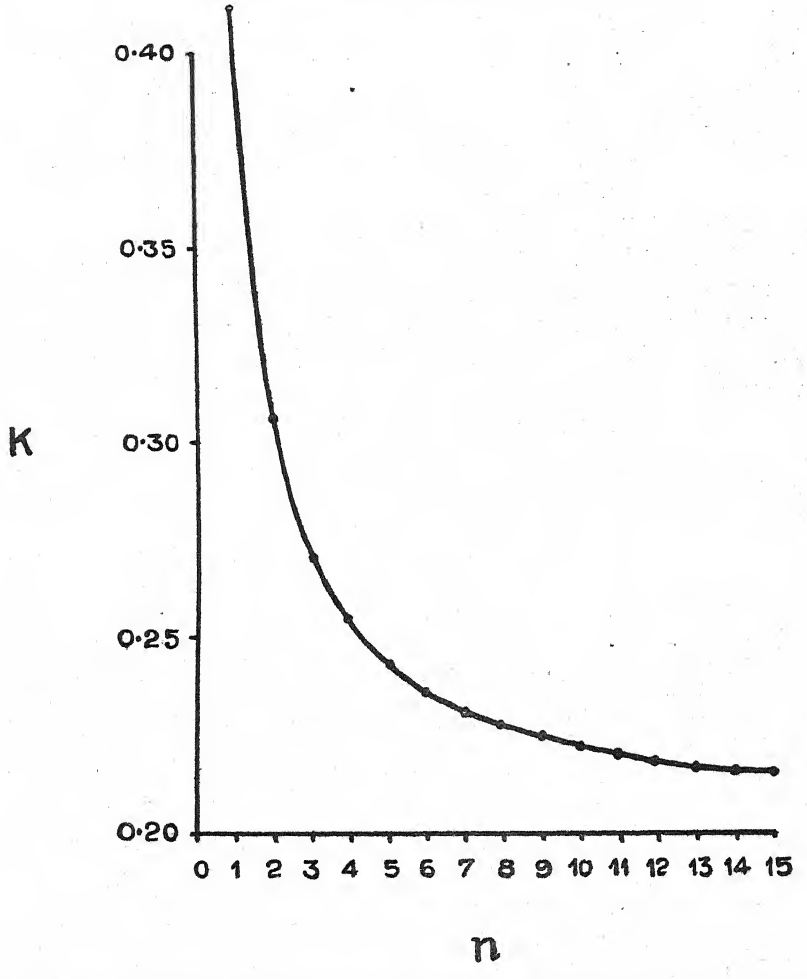


Fig. 1. Effect of changes in number of measurements per sample on variance of mean of six samples. Calculated from Dhar data.

Any increase in the number of measurements above ten reduces the variance of the mean (= K) very little, and a decrease in n from ten to five only increases K from 0·223 to 0·244, an increase of just over 9 per cent in variance, which corresponds to an increase of 4·3 per cent in standard error. Further reductions in n lead to a rapid increase in K.

For practical purposes in variety testing, a significant difference of 1·5 mm. is all that is required. For such a difference to be significant, the standard error of the difference must be 0·75 mm. or less, corresponding to a standard error of the mean of 0·515 mm. or less. K will then be $(0{\cdot}515)^2$ or 0·265. From Fig. 1, $K = 0{\cdot}265$ when n is about 3·5. At that point the value of K is increasing rapidly with decreases in n. Five measurements per sample should, however, give a

standard error below the required limit and ensure that sampling errors contribute only a small amount to it. Five experiments have been studied in the current season in each of which seven Indian varieties from different parts of India were included. Five measurements were made per plot on each of six plots per variety, and the following standard errors of the means were obtained :—

0·45, 0·39, 0·35, 0·44, 0·49,

giving significant differences from 1·0 mm. to 1·4 mm. Two other experiments were studied in which two Indian varieties were compared with an acclimatised American variety. Five measurements per plot were made on each of eight plots per variety, and standard errors of 0·50 mm. and 0·52 mm. were obtained. Agreement with expectation was, therefore, good in all cases.

In order to estimate the sampling error of halo-length determinations on single-plant samples, halo-length data were examined from 50 plants grown in an Indian cotton selection experiment. Five measurements were made on each plant. The variance was analysed into sampling variance and variance due to differences between plants. Results are given below :—

	Degrees of freedom	Mean square
Between plants	49	8·1250
Sampling	200	0·6162

Variance (mean of 5) = 0·1232

σ (mean of 5) = 0·351 mm.

The mean of 5 measurements per plant gives a sufficiently accurate estimate of halo-length for practical purposes. Comparing the single-plant data with the plot data from Dhar and Limbodi, it appears that about half the sampling error in plot samples may be ascribed to differences from seed to seed on the same plant.

All the measurements above quoted were made by one of us (V. G. Panse), or by one of our colleagues (N. S. Apte) on seeds combed by girls trained for the purpose. For making large numbers of halo-length measurements intelligent boys, literate in English, have been trained, and it was decided to investigate the standard error of their measurements and compare them with our own. Sets of five measurements on each of 50 plants were examined, as above. Below are given sampling variances for four observers, and the number of measurements required to give a variance of the mean equal to or less than 0·25 (corresponding roughly to a significant difference between means of 1·5 mm.) :—

Observer	Sampling variance	No. of observations for V (M) = 0·25 or less
K. G. (American)	3·702	15
(Indian)	3·026	12
D. U.	1·541	6
Y. U.	1·094	5
D. L.	2·450	10

No observer has reached the standard of accuracy of our own figures, but the results obtained by D. U. and Y. U. are reasonably satisfactory. Since all observations were taken on very similar material, except those on American cotton by K. G., observers K. G. and D. L. must be judged definitely inferior to D. U. and Y. U.

The results obtained by K. G. on American and Indian cottons show that the variability is of the same order in both.

Discussion

Bailey [1930] has described in detail the method of determining halo-length employed in the Sudan. While it is the primary purpose of this paper to discuss the error to which such measurements are subject, it is necessary to refer to the method of measurement employed. Combing is done in the way described by Bailey, but with a steel weaver's comb. A xylonite comb is not suitable for combing Indian cottons. Measuring is done with Bailey's celluloid staple-length measurer, but only the maximum halo-length is recorded. The edge of the halo is taken as the end point, and not the ends of stray hairs, which may be detached from the seed.

There does not appear to be anything to be gained by taking five measurements per seed, and reducing the average by a small amount to compensate for the shorter hairs at the base of the seed, as advocated by Bailey. In any case halo-length can never be an estimate of mean hair-length, as the vast majority of the shorter hairs must always be hidden by the longer ones. Since it must be an estimate of maximum hair-length, for facility of measurement and ease of training of staff, it should be as simple as possible.

Taylor [1930] states that in Nigeria, to obtain a degree of accuracy of 0·5 mm., it is necessary to comb and measure 50 seeds per plant. If by " degree of accuracy " is meant standard error of the mean, under the conditions of these experiments, five measurements per plant should be adequate with properly trained observers. For a significant difference of 0·5 mm., 20 to 25 measurements should be sufficient. It does not seem likely that under any conditions there will be much to be gained by aiming at a significant difference of less than 1 mm. For all ordinary purposes a significant difference of 1·5 mm. will be sufficient, and with properly trained observers this should be attained by taking the mean of five measurements per sample.

Acknowledgments

We are indebted to Mr. N. S. Apte for the use of halo-length measurements made by him.

The material from which the Dhar and Limbodi data were obtained was supplied by the Superintendent of Agriculture, Dhar State, and the Registrar of Co-operative Societies, Holkar State, respectively, and we take this opportunity of offering them our thanks.

SUMMARY

1. Information was collected on halo-length, plot by plot, in replicated variety trials. Combing was done by girls trained for the purpose, and measurements were made by skilled observers.

2. Similar data was collected on halo-lengths of single plants. Combing was done by the same girls, but some sets of measurements were made by semi-skilled laboratory assistants.

3. It is shown that with skilled observers, five measurements per plot, or per plant, are adequate to give a standard error of the mean of 0·5 mm.

4. Laboratory assistants varied greatly in their efficiency as measured by the standard error of the means of their observations, indicating the necessity of careful training and periodical checking of such subordinate staff.

5. In replicated plot trials with measurements by skilled observers, within-plot variation was responsible for only a small proportion of the error variance. Sampling for staple-length measurement should, therefore, be plot-wise, and not from the pooled product of all plots of a variety.

REFERENCES

Bailey, M. A. (1930). Report and Summary of Proc., E. C. G. C. Conference on Cotton Growing Problems, p. 152.

Immer, F. R. (1932). *J. Agric. Res.* **44**, 633.

Taylor, C. B. (1930). Report and Summary of Proc., E. C. G. C. Conference on Cotton Growing Problems, p. 157.

A Note on the standard error of estimates of ginning percentage in variety trials

It is not usually possible to arrange for the ginning of the produce of individual plots separately in variety trials, and there appear to be no records available from which an estimate can be made of the reliability of ginning percentages determined from ginning records of the total produce per variety in such trials.

The produce of five cotton variety trials, laid out in randomised blocks in the 1934-35 season, was ginned plot by plot and ginning percentages were treated by the method of the analysis of variance. The table below gives mean squares, standard errors of the mean, and significant differences for these experiments.

Analysis of variance of ginning percentage

	2 pairs of blocks		Six blocks					Eight blocks		
	Barwani			I. P. I. medium land		I. P. I. poor land			I. P. I. medium land	poor land
	Degrees of freedom	Mean square	Degrees of m	Pickings 1 and 2	3rd picking	Pickings 1 and 2	3rd picking	Degrees of freedom	Mean square	Mean square
				Mean square	Mean square	Mean square	Mean square			
Blocks . .	2	0·20	5	0·13	1·17	2·6	0·61	7	1·65	0·36
Varieties . .	6	89·97	6	142·70	110·26	83·97	88·45	2	10·85	14·58
Error . .	12	0·36	30	0·18	0·65	4·24	1·16	14	1·14	0·11
Standard error of mean	...	0·30	...	0·17	0·33	0·84	0·44	...	0·38	0·12
Significant difference	...	0·90	...	0·50	0·96	2·40	1·33	...	1·00	0·35

As in the case of staple-length, differences between blocks were never greater than would be expected on random sampling only.

In all cases except Institute of Plant Industry poor land, a difference of one per cent in ginning outturn was significant. In the Institute of Plant Industry poor land experiment the total yield was very low, and 1st and 2nd pickings contributed only a small part of it. The land used in this experiment was very uneven and there were large differences in yield between plots.

Summary

Where it is not possible to gin plot yields separately, the data here presented suggest that, provided the experiment was not carried out on very uneven land, a difference in ginning outturn of one per cent may be regarded with some confidence as real.

STUDIES IN THE TECHNIQUE OF FIELD EXPERIMENTS

III. AN APPLICATION OF THE METHOD OF COVARIANCE TO SELECTION FOR DISEASE RESISTANCE IN COTTON

BY

J. B. HUTCHINSON

AND

V. G. PANSE,

Institute of Plant Industry, Indore, Central India

(Received for publication on 19th June 1935)

The analysis of covariance is being applied increasingly to the study of the effect of treatments on the yield of perennial crops. For this purpose plot yields are taken in a pre-experimental period, and the experimental yields are adjusted for the regression of experimental on pre-experimental yields [Eden, 1931; Murray, 1934]. Up to the present the method has not been of much use to workers with annual crops, since as great an advantage is not obtainable when it is not possible to use the same plants in the control and experimental periods. Such experience as is available indicates that a uniformity trial with an annual crop is not likely to increase the accuracy of the succeeding experiment by more than 60 per cent. For this increase the labour expended is doubled and the acquisition of the result postponed by a year [Fisher, 1934].

In the experiment to be described a uniformity test was superimposed on a variety trial. The loss of a season was thereby avoided, and the accuracy of the comparison between variety and uniformity plots increased by the elimination of seasonal differences.

In the course of a study, by the Baroda State Department of Agriculture, of methods of avoiding the damage to the cotton crop of Gujerat by root-rot disease, the authors were consulted as to the best method of testing the disease resistance of a number of selections from Gujerat varieties and from cultivators' fields in affected districts. Only a limited amount of land known to be heavily infected with the disease was available for experimental purposes, and inspection of data from non-replicated progeny row tests carried out in 1933-34 led to the conclusion that considerable variations in the intensity of attack might be expected within the area.

For the 1934-35 crop the material was divided into two experiments:—

(1) Strain No. 8 (A)
Strain No. I 32/24 (B)
Strain No. I 65/12 (C)
} Selections from the standard variety Broach 9.

and

(2) District samples (F)
Broach 9 survivals (G)
Karakhadi survivals (K)
} Survivors from root-rot infested fields.

Rozi and Broach 9, two Gujerat varieties, were included in both experiments. Each experiment was laid out in two 5×5 Latin squares, with 24 ft. $\times$ 25 ft. plots. In each plot there were 8 rows 25 ft. long, each row containing 25 plants one foot apart. Of the 8 rows, alternate rows were sown with the susceptible control variety, Broach 9, giving four rows of control and four rows of the strain under test in each plot. In Broach 9 plots, of course, all 8 rows were of the same variety. Two rows of Broach 9 were sown all round the experiment as guard rows. The experiments were sown in May, six weeks before the onset of the monsoon, as it is known that the intensity of root-rot attack is much higher with early-sown than with rain-sown crops.

Deaths from root-rot were recorded row by row at intervals throughout the crop period. At the conclusion of the experiment percentage deaths were calculated separately for the control and varietal rows in each plot.

The rows of Broach 9, distributed in each plot in the same way as the rows of the strain under test, were expected to sample the same intensity of the disease as the strain, and the plot-to-plot variation in mortality in the strains was therefore adjusted for the mortality in the control rows by the application of the analysis of covariance.

The analysis of the results was carried out in four stages:—

(1) Analysis of variance of percentage deaths in variety rows in all plots.

(2) Analysis of variance of percentage deaths in control (Broach 9) rows in all plots.

(3) Analysis of covariance of percentage deaths in variety and control rows in the same plots.

(4) Analysis of variance of percentage deaths in variety rows adjusted by the covariance method for the mortality in control rows.

In Table I are given mean squares for error and for variety differences before and after adjusting for the covariance of control and variety mortality.

TABLE I

Crude and adjusted mean squares due to error and to variety differences

Latin square	Mean percentage mortality in control	Error mean square		Variety mean square		Standard error percentage of mean	
		Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
I-a . . .	65·3	179·0	98·6	1824·2	1559·5	11·6	8·6
I-b . . .	63·5	151·9	68·8	1328·2	818·2	11·3	7·6
II-a . . .	71·9	84·3	44·4	3305·7	2373·2	8·2	5·9
II-b . . .	45·4	119·7	88·5	698·2	714·0	15·6	13·4

In squares I-a, I-b, and II-a the mean square for error is reduced by the adjustment to approximately half the crude value. In square II-b the reduction in mean square is only about 25 per cent. In the first three squares the mean mortality in Broach 9 control rows was from 63 to 72 per cent, while in square II-b it was only 45 per cent. The efficiency of the method of covariance in reducing the error mean square depends upon the accuracy with which the control rows sample the disease intensity to which the variety rows are exposed. The data from square II-b indicate that with a lower disease intensity the agreement between variety and control rows is considerably reduced. This is illustrated by dividing the error sum of squares before adjustment into the parts contributed by: (1) regression of variety mortality on control mortality and (2) deviations from regression. Figures are given in Table II.

TABLE II

Analysis of error sum of squares

	Degrees of freedom	Latin square							
		I-a		I-b		II-a		II-b	
		Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square
Regression . . .	1	1063	1063	1066	1066	524	524	463	463
Deviations from regression	11	1085	98·6	757	68·8	488	44·4	974	88·5
Crude error . . .	12	2148	...	1823	...	1012	...	1437	...

In squares I-a, I-b, and II-a half or more than half of the original error sum of squares is accounted for by regression and half or less than half by deviations from regression, whereas in square II-b only about one-third of the original sum of squares is absorbed by regression, leaving two-thirds to deviations from it. The regression coefficients are in all cases significant.

When the experiments were laid out in pairs of Latin squares it was intended to combine the data from squares containing the same strains in order to improve the accuracy of comparison. Unfortunately, owing to an error, the same randomizations were used for both squares in the same pair, so that combination of the data is not strictly permissible. In order to obtain a comparison of the relative efficiency of doubling the number of plots, and of using the covariance of control rows with variety rows, in reducing the error variance, combined analyses were, however, calculated. Figures for standard errors per cent of means of five plots (variety rows only) in single squares and of ten plots (variety rows only) in combined squares are given below :—

Latin square	Mean of 5 plots		Mean of 10 plots
	Not adjusted	Adjusted	Combined not adjusted
I-a	11·6	8·6	8·1
I-b	11·3	7·6	
II-a	8·2	5·9	7·8
II-b	15·6	13·4	

The covariance method has, on the average, given approximately the same statistical advantage as doubling the number of plots. It gives an advantage not given by doubling the number of plots in providing a local control where the attack of the disease is distinctly patchy. With independent randomizations in the pairs of squares, advantage could have been taken of both methods of reducing error.

The method of distributing control rows of a common variety through a series of variety plots is one to be used with discretion. Quite clearly, it cannot be used in yield-tests where varieties of considerably different habits of growth are under trial. It appears to have, however, very definite possibilities in testing a number of strains of rather similar growth habit, especially for characters,

such as disease resistance. It should have a particular appeal to the plant-breeder, who is often confronted with the need for making accurate comparisons between strains of which he has very limited amounts of seed.

Summary

An experiment in an investigation of the resistance of cotton strains to root-rot disease is described, in which alternate rows of all plots were sown with a uniform control strain.

Details are given of the application of the method of covariance to the data obtained.

The error variance was in general reduced to about one-half by adjustment for the covariance of control and variety rows, giving the same advantage as would be obtained by doubling the number of plots.

The scope and advantages of the method are briefly discussed.

Acknowledgments

The experiment here reported was designed at the Institute of Plant Industry, Indore, and carried out at Baroda as part of the programme of the Cotton Root-Rot Scheme conducted by the Department of Agriculture, Baroda State, with the aid of a grant from the Indian Central Cotton Committee. We are indebted to the Director of Agriculture, Baroda State, and the Indian Central Cotton Committee for permission to use the data, and to the Officer-in-charge, and the Plant Breeder, Cotton Root-Rot Scheme, for their co-operation.

REFERENCES

Eden, T. (1931). *J. Agric. Sci.* **21**, 547.

Fisher, R. A. (1934). "Statistical Methods for Research Workers", 5th Edition. Oliver and Boyd, London.

Murray, R. K. S. (1934). *J. Agric. Sci.* **24**, 177.

A PRELIMINARY NOTE ON THE CLASSIFICATION OF CULTIVATED INDIAN MUSTARDS

BY

T. S. SABNIS, B.A. (Hons.), M.Sc., I.A.S.,

Economic Botanist to Government, United Provinces,

AND

M. G. PHATAK, L.Ag. (Bom.),

Research Assistant

(Received for publication on 14th May 1935)

CONTENTS

I. Introduction

The observations embodied in this paper are mainly confined to the classification of the cultivated Indian mustards into agricultural types. While examining the botanical characters of the cultivated Indian mustards grown from seeds from different parts of India in connection with their breeding work on the crop, the authors felt the necessity of such a classification. The previous workers had either touched only their botanical characters or grouped them into agricultural types in an imperfect manner.

Prain [1896], in his note on the mustards cultivated in Bengal, dealt with their botanical aspect only and classified them into species and varieties. His classification is valuable and is the only one of its kind on cultivated mustards. As it is, however, confined mainly to the crops grown in Bengal—the area now covered by the provinces of Bengal and Bihar and Orissa—it fails to be of much help in the classification of mustards cultivated in India as a whole. Besides, being a purely botanical classification, it cannot be used with advantage in the identification and isolation of numerous agricultural types which are so very common in *rai* and *sarson*.

Howard, Howard and Khan [1915] have discussed the biology of the mustards, and their observations form a valuable contribution to the study of this crop. Their classification of *Brassica juncea* into agricultural types affords useful information ; but at the same time it clearly shows the difficulties of grouping all the cultivated Indian mustards into a definite scheme. They have remarked that " the labour of reducing them (mustards) to paper is so great that no effort has been made to accomplish this exceedingly difficult task ". The authors are fully conscious of these difficulties and feel rather diffident of the result of their attempt. They have however endeavoured to present, in this paper, a scheme of classification which they hope will be helpful to the workers on this crop.

The authors obtained from different parts of India a large collection of seed samples of *B. nigra* Koch (Benarasi *rai*), *B. rugosa* Prain (Pahadi *rai*), *B. juncea* H. f. & T. (*rai*), *B. campestris* L. (*sarson*), *B. napus* L. (*tori*), and the pure lines isolated from the plants grown from these samples provided them with sufficient material for their work.

In this work, the classification of Prain has been fully taken into account and the changes that have been made in his nomenclature of varieties are with a view to make their names more suggestive of the principal distinguishing characters.

The authors are indebted to Messrs. C. E. Parkinson and M. B. Biswas for facilities to study the herbarium specimens of mustards at the Forest Research Institute, Dehra Dun, and the Royal Botanical Gardens, Calcutta, respectively, to Dr. F. J. F. Shaw for criticism and advice and to Dr. S. P. Agharkar and Mr. P. K. Dey for helpful suggestions.

II. General plan of classification

Five species of cultivated Brassicas—*B. nigra* Koch, *B. rugosa* Prain, *B. juncea* H. f. & T., *B. campestris* L. and *B. napus* L.—are dealt with in this note.

B. nigra (Benarasi *rai*) and *B. napus* (*tori*) are almost self-sterile and widely cross-pollinated in nature. Among the plants raised from the seed samples of these two species, received from different parts of India, differences pertaining

to height and maturity, shape, size and colouration of the leaves, mode of branching, size and colour of the flowers were well marked; but owing to their self-sterility and cross-pollination under natural conditions it was not possible to fix varieties or agricultural types of these species.

B. rugosa (Pahadi *rai*) is grown as a vegetable in the Kumaon hills. Its seed, it is reported, is also crushed for oil on a small scale for local consumption; but it does not enter the trade in oil-seeds. As the crop is not of any importance in the agricultural industry, no attempt was made to fix its varieties or agricultural types.

B. juncea (*rai*) has been divided on the basis of hairs into two varieties—*Hirsuta* and *Glabra*. These are further subdivided into eleven agricultural types, eight under *Hirsuta* and three under *Glabra*, based on the time of maturity, height, branching, form of the leaf and colour of the seed-coat.

B. campestris L. (*sarson*) has been divided on the basis of the habit of the fruit into two varieties—*Erectisiliquosa* with ascending pods and *Pronisiliquosa* with pendant pods. Each of these varieties is subdivided into two groups—(1) 2-valved and (2) 3-4-valved. These groups are further classified on the basis of height, presence or absence of hairs on the stem and leaf, colour of the flower, shape of the fruit and colour and markings of the seed, into agricultural types—thirty-two under *Erectisiliquosa* and three under *Pronisiliquosa*. The *Pronisiliquosa* Type 33 with 2-valved pendant pods is based on the herbarium specimens from the Royal Botanical Gardens, Calcutta. It has been included in this note to make this classification somewhat complete.

III. Key to the mustards

I. Pods appressed to the axis . . . . . *B. nigra.*

II. Pods spreading or semi-appressed to the axis.

A. Pods cylindrical and beaded; and leaves tapering to the base.

(*a*) Leaves at the base persisting and forming a cluster.
Stem scape-like . . . . . *B. rugosa.*

(*b*) Leaves at the base withering and not clustered.
Stem normally branched . . . . . *B. juncea.*

(1) Leaves hairy . . . . . *B. juncea* var. Hirsuta.

i. Pods spreading.

(i) Flowers lemon-yellow.

(*a*) Margins of leaves green.

Tall, late . . . . . Type 1.

Tall, intermediate in maturity . . Type 2.

Intermediate in height and maturity . Type 3.
Intermediate in height but early . . Type 4.
Short, early . . . . . Type 5.

(β) Margins of leaves purplish.
Short, early . . . . . Type 6.

(ii) Flowers picric-yellow.
Short, early . . . . . Type 7.

ii. Pods semi-appressed to the axis.
Short, early . . . . . Type 8.

(2) Leaves glabrous . . . . . *B. juncea* var. *Glabra.*

i. Pods spreading; flowers lemon-yellow; margins of leaves green.

Tall, late . . . . . Type 9.
Intermediate in height and maturity . Type 10.
Intermediate in height but early . . Type 11.

B. Pods ascending or pendant, 2-4-valved. Stem-leaves auricled and stem-clasping . . . . *B. campestris.*

(*a*) Pods ascending . . . . . *B. campestris* var. *Erectisiliquosa.*

(1) Pods flat and 2-valved.

i. Flowers yellow; seed yellow.

(i) Plants hairy.

(*a*) Tall and late in maturity.

Lamina portion connecting the terminal lobe and uppermost lateral lobes folded inwards . . . . . Type 1.

Lamina portion connecting the terminal lobe and uppermost lateral lobes broad and flat . . . . . Type 2.

(β) Tall and intermediate in maturity.

Lamina portion connecting the terminal lobe and uppermost lateral lobes folded inwards . . . . . Type 3.

Lamina portion connecting the terminal lobe and uppermost lateral lobes broad and flat . . . . . Type 4.

(γ) Intermediate in height and late in maturity.

Lamina portion connecting the terminal lobe and uppermost lateral lobes flat . Type 5.

Lamina portion connecting the terminal lobe and uppermost lateral lobes irregular . . . . . Type 6.

(δ) Intermediate in height and maturity.

Lamina portion connecting the terminal lobe and uppermost lateral lobes narrow and flat . . . . . Type 7.

Lamina portion connecting the terminal lobe and uppermost lateral lobes broad and flat . . . . . Type 8.

(ε) Short and early in maturity.

Lamina portion connecting the terminal lobe and uppermost lateral lobes broad and flat . . . . . Type 9.

(ii) Plants glabrous.

Tall, late . . . . . Type 10.
Intermediate in height and maturity . Type 11.
Intermediate in height, early . . . Type 12.
Intermediate in height, late . . . Type 13.
Short, early . . . . . Type 14.

ii. Flowers yellow ; seed brown.

(α) Pods broad.

Intermediate in height and maturity . Type 15.
Short, intermediate in maturity . . Type 16.
Short, early . . . . . Type 17.

(β) Pods narrow.

Short, intermediate in maturity . . Type 18.

iii. Flowers naphthalene-yellow ; seed brown.

Short, early . . . . . Type 19.

Intermediate in height, late . . . Type 20.

(2) Pods cylindrical and 2-valved.

i. Flowers lemon-yellow ; seed yellow.

Intermediate in height, early . . . Type 21.

ii. Flowers yellow ; seed brown.

(i) Leaves at the base not clustered. Stem normally branched.

(α) Plants hairy.

Late in maturity. Pods not beaded . Type 22.

Intermediate in maturity. Pods slightly beaded . . . . Type 23.

(β) Plants glabrous.

Intermediate in maturity . . . Type 24.

(ii) Leaves at the base clustered. Stem scape-like.

Intermediate in height, late . . Type 25.

(3) Pods angled and 3-4-valved.

i. Flowers yellow ; seed yellow.

(i) Late in maturity.

Pods much inflated . . . . Type 26.

(ii) Intermediate in maturity.

(α) Beaks medium.

Pods slightly inflated . . . Type 27.

Pods very much inflated . . . Type 28.

(β) Beaks long.

Tall ; pods slightly inflated . . . Type 29.

Intermediate in height ; pods much inflated . . . . . Type 30.

(γ) Beaks short.

Intermediate in height ; pods much inflated . . . . . Type 31.

ii. Flowers naphthalene-yellow ; seed yellow.

Short, early . . . . . Type 32.

(*b*) Pods pendant . . . . . *B. campestris* var. *Pronisiliquosa.*

(1) Pods flat and 2-valved.

i. Flowers yellow ; seed yellow.

Short, early . . . . . Type 33.

(2) Pods angled and 3-4-valved.

i. Flowers yellow ; seed yellow.

Tall, late . . . . . Type 34.

Short, early . . . . . Type 35.

C. Pods ascending, 2-valved, cylindrical and beaded. Stem-leaves auricled and stem-clasping . . *B. napus.*

IV. Description of species and their varieties

Brassica nigra Koch (*Benarasi rai, Mohari, Asal rai, Multani rai*)

This is a cold-weather crop grown to some extent in the United Provinces, Madras, Punjab and the North-West Frontier Province. Plants erect or bushy, very small-seeded, self-sterile, maturity of seed between 130 and 165 days. Height 120-250 cm. Stem slightly glaucous and green, branching usually above the base. Axillary angle between branches and the main axis 35° decreasing upwards to 20°. Stem-leaves non-stem-clasping, stalked, bristly hairy, lobed or pinnatisect, with lobes further incised in some cases, forming a cluster at the base, light or deep green, midrib white or purplish, margins undulated and dentate, 13-36 cm. long and 8-11 cm. wide. Leaves on floral shoots stalked, hairy, lanceolate, margins entire, 12-5 cm. long and 18-5 mm. wide. Inflorescence corymb, elongating into a raceme 50 cm. or more long. Bracts and bracteoles present in some plants. Pedicel 6-8·5 mm. long. Calyx light green and spreading in the young condition and picric-yellow and closed in the opened flowers. Sepals boat-shaped and hooded, 4 mm. long and 1 mm. wide. Corolla 1·2-1·5 mm. long, membranous and with a yellow midrib. Limb obovate or rounded with or without a notch at the apex, 4-5 mm. long and 3-4 mm. wide. Petals distinctly less broad than in other species. Stamens distinctly overtopping the stigma which just touches the base of the anthers. Outer stamens 4-5 mm. long. Inner stamens 5-6 mm. long. Ovary 2-3 mm. long. Style 1-1·5 mm. long. Stigma lemon-yellow and broad. Fruits cylindrical, beaded, 2-3 cm. long including stalk and beak, and appressed to the axis. Stalk 4-6 mm. long and beak 1-2 mm. long. Seed very small, ovoid, faintly rugose, brown.

Brassica rugosa Prain (*Pahadi rai*)

Grown in the cold weather as a vegetable in the Kumaon hills. Root slender and tapering. Stem scape-like, stout, 100-125 cm. high with branches as high and forming a pyramidal head 25-35 cm. across. Radical leaves forming a cluster, ovate, narrowed to the base, not stem-clasping, thick, crumpled, green and glossy, bristly hriry, 40-70 cm. long, 14-30 cm. broad; midrib white, thick, deeply channelled; margins broadly dentate. Cauline leaves similar to radical leaves in shape but smaller in size, and tapering to a stalk-like condition towards the base, not stem-clasping, decreasing in size upwards. Inflorescence corymb, elongating into a raceme 5-10 cm. long in the flowering condition and increasing to 15-30 cm. during pod formation. Pedicels 5-7 mm. long, elongating to 1 cm. after fertilization. Sepals light green when young and changing to pale lemon-yellow before falling, sub-erect, boat-shaped, hooded, 5-6 cm. long and 1 mm. broad. Corolla lemon-yellow, 1 cm. across. Petals 6-6·5 mm. long. Claw 3-3·5 mm. long. Limb 2·5-3 mm. long and 2-3·5 mm. broad. Fruit 4-4·5 cm.

long, cylindrical, 2-valved, beaded, containing 10-16 seeds ; axillary angle between the pedicel and the floral axis 20°-30° ; stalk 7-10 mm. long ; beak 7-8 mm. long. Seed small, ovoid, brown, rugose, hilum white.

Brassica juncea H. f. & T. (*Gohna-sarson, Badshahi-Lai, Khas-rai*)

Root very stout and tapering. Leaves lyrately-lobed and narrowed to the base and not stem-clasping. Basal leaves quickly withering, green and a little glaucous. Stem elongating from the commencement of the growth and with purplish tinge at the nodes. Flowers lemon-yellow. Pods cylindrical, beaded, smooth, spreading or semi-appressed to the stem. Beak short. Seed dark brown or reddish brown or red, small and rugose.

Brassica juncea var. *Hirsuta* (*Nova*)

A cold-weather crop generally cultivated mixed with other crops throughout India. Height 75 to over 250 cm. Stem glaucous and green ; branched from the very base or little above the base ; axillary angle between branches and the main axis 45° decreasing upwards to 30°. Stem-leaves hairy ; stalk stout, broadened at base, furrowed on the upper surface and cylindrical below, upto 8·5 cm. long ; sinnuate lyrate, lobed or pinnatisect, lobes very unequal ; margin broadly toothed or serrate and uneven ; apex obtuse ; midrib white and slightly furrowed on the upper surface ; 27-49 cm. long and 15-20 cm. wide. Leaves on floral shoots stalked, small, triangular, lanceolate ; margin entire ; apex acute ; 11·5-2 cm. long and 7-1·5 cm. wide. Inflorescence corymb, 1-2·2 cm. long. Sepals light green and closed in buds, and yellow and spreading in the opened flowers, boat-shaped and hooded, 7 mm. long and 1·5 mm. wide. Corolla 1·7-1·8 cm. across. Claw membranous with a conspicuous yellow midrib, 4-5 mm. long. Limb 7-8 mm. long and 6 mm. wide, lemon-yellow. Outer stamens 6 mm. long and inner stamens 8 mm. long. Anthers recurved after bursting. Ovary 5 mm. long, green ; style 2 mm. long, green ; stigma lemon-yellow and capitate. Fruit spreading, 6·2 cm. long including stalk and beak, beaded, and cylindrical. Stalk 1·7 cm. long and beak 1·0 cm. long. Seed spherical or ovoid, smaller than that of *sarson* distinctly rugose, brown, deep brown or black.

Brassica juncea var. *2 Glabra* (*Nova*)

A cold-weather crop, generally cultivated mixed with other crops in most of the provinces in India. Height 135-205 cm. Stem glabrous, glaucous, green with or without a tinge of purple, branching from or above the base. Angle of branching 45° decreasing upwards to 30°. Branches more or less of equal length. Stem-leaves stalked, glabrous, green, sinnuate lyrate, 12-45 cm. long and 4-18 cm. wide ; margins broadly serrate ; midrib grooved and purplish sometimes.

Leaves on floral shoots glabrous, oblong-lanceolate to linear-lanceolate, margins entire, petiole with narrow wings in continuation of the lamina, 5·2-5 cm. long and 7-1·5 cm. wide. Inflorescence corymb, elongating into a raceme up to 60 cm. long. Pedicel up to 1 cm. Calyx light green and closed when young, and pale lemon-yellow and spreading in opened flowers. Corolla lemon-yellow, 1·5-1·7 cm. across. Claw 4 mm. long, membranous and with yellow midrib. Limb 7-8 mm. long and 7 mm. wide. Outer stamens 6 mm. long. Inner stamens 8 mm. long. Ovary 3-3·5 mm. long. Style 2-3·5 mm. long. Stigma lemon-yellow, broad and rounded, and flush with anthers in opened flowers before the anthers burst. Fruit, spreading, 5-6 cm. long including beak and stalk and 4 mm. broad, ridged along the replum and elliptical in cross section. Stalk 1·5 cm. Beak 9-11 mm. long. Seeds of the same size as in Hirsuta and smaller than that of *sarson*, ovoid, brown, with distinct black spot at the hilum and very rugose.

Brassica campestris L. (*Sarson, Pivli rai, Banga sarson, Shwet rai, Bara sarson, Pila sarson*)

Root stout and tapering; leaves glaucous, dark green and hairy beneath; cauline leaves auricled and stem-clasping; radical leaves smaller than cauline leaves and narrowed to the base and not stem-clasping; stem elongating from the commencement of growth and with purplish tinge in some cases, branching starting usually from the base; pods stout, 2-4-valved and spreading or semi-appressed; beak stout but short; seed brown or yellow, smooth and bold.

Brassica campestris L. var. *1. Erectisiliquosa* (*Nova*), *2-valved*

Plants belonging to this variety formed the major portion of the crop raised from *sarson* samples received from different parts of India and represent the *sarson* crop in the United Provinces. Height 75-175 cm. Stem glabrous or hairy, glaucous, green, branching from 6-10 cm. above the base. Stem-leaves glabrous or hairy, 28-18 cm. long and 15-4 cm. wide; sinnuate lyrate, dark green, stem-clasping; auricles rounded and extending half way on the opposite side of the stem; margins dentate. Leaves on floral shoots glabrous, glaucous, dark green, triangular-lanceolate, stem-clasping, 11·5 cm. long and 3·5-2 cm. wide; auricles rounded, surrounding the stem and overlapping; margins entire. Inflorescence corymb, elongating into a raceme. Flower stalked; pedicel 1·2 cm. long. Sepals keel-shaped, hooded, green and spreading when young and pale lemon-yellow and closed in opened flowers, 5 mm. long and 1·5 mm. wide. Corolla 1·5 cm. across. Claw membranous and pale lemon-yellow, 3 mm. long. Limb lemon-yellow, 7 mm. long and 5 mm. wide. Filaments dull green, 6 mm. long in the outer stamens and 8 mm. long in the inner stamens. Anthers yellow and recurved after bursting. Ovary stalked, style green and stout, stigma lemon-yellow. Fruit 2-valved, ascending flat or cylindrical, 12·5

cm. long including stalk and beak. Stalk 4·5 cm. long, and beak 2·5 cm. long. Seed ovoid, yellow or brown, large or small, smooth or faintly rugose, hilum distinct.

Brassica campestris. L var. *1. Erectisiliquosa (Nova), 3-4-valved*

Numerous specimens of this variety were found amongst plants raised from *sarson* seed samples which were received from the United Provinces, Bihar and Bengal. Height 75-175 cm. Stem glaucous, green, branching from the base. Stem-leaves hairy or glabrous, dark green, lyrately pinnatipartite, stem-clasping, 36-13 cm. long and 12-4 cm. wide; auricles rounded and extending half-way on the opposite side of the stem; margins broadly dentate. Leaves on floral shoots glaucous, dark green, glabrous, triangular-lanceolate, stem-clasping, 11-5 cm. long and 3·5-2 cm. wide; auricles rounded, surrounding the stem, and overlapping; margins entire. Inflorescence corymb, elongating into a raceme, 18 cm. long before pod formation and extending to 60 cm. after full development of the pods. Flower stalked; pedicel 1·5-1·8 cm. Corolla 1·75 cm. across. Sepals keel-shaped and hooded, light green and spreading when young, pale lemon-yellow and closed when fading; 7·5 mm. long and 2 mm. wide. Claw pale lemon-yellow, 5 mm. long; limb lemon-yellow, 6 mm. long and 5 mm. wide. Stamens slightly overtopping the stigma; filaments light green, 6 mm. long in the outer stamens and 7 mm. long in the inner stamens. Anthers yellow and recurved after bursting. Ovary stalked; style green and stout; stigma lemon-yellow. Fruit spreading and 3-4-valved, 12 cm. long including stalk and beak, stalk 5 cm. long and beak 2·5 cm. Seed large, ovoid, smooth, yellow; hilum distinct.

Brassica campestris L. var. *Pronisiliquosa (Nova), 2-valved*

Height 130-175 cm. Stem glabrous, glaucous, green, branching from the base. Stem-leaves glabrous, sinnuate-lyrate, dark green, stem-clasping; 28-18 cm. long and 15-4 cm. wide; auricles rounded and extending half-way on the opposite side of the stem; midrib on the upper surface, white with its lower portion channelled; margin broadly dentate with teeth blunt. Leaves on floral shoot oblong-lanceolate or lanceolate, glabrous, glaucous, dark green and stem-clasping; auricles rounded, surrounding the stem and overlapping. Inflorescence corymb, elongating into a raceme. Flower stalked; pedicel 1·2 cm.; sepals keel-shaped and hooded, green and sub-erect in young condition, and pale lemon-yellow and spreading in opened flowers. Corolla 1·5 cm. across. Petals clawed; claw membranous, pale lemon-yellow, 3-5 mm. long; limb lemon-yellow, 7-8 mm. long and 5 mm. wide. Filaments of outer stamens 6 mm. long and of inner stamens 7 mm. long. Anthers 2 mm. long. Ovary stalked. Style green, cylindrical and stout, 2 cm. long. Stigma lemon-yellow and capitate. Fruit 2-valved, flat, pendant, 11 cm. long including beak and stalk and 7 mm. wide. Stalk 2 cm. long; beak 3 cm. long. Seed large, ovoid, yellow and smooth; hilum distinct.

Brassica campestris L. var. *Pronisiliquosa* (*Nova*), *3-4-valved*

Numerous specimens of this variety were found amongst plants raised from *sarson* seed samples received from the United Provinces and Bihar.

Flower stalked, pedicel up to 2·3 cm. Sepals 5 mm. long and 1 mm. wide. Corolla 1·4 cm. across. Petals 8 mm. long; claw membranous with central portion yellow; limb lemon-yellow, 5 mm. long and 4 mm. wide. Fruit pendant, 3-4-valved, up to 10-12 cm. long including stalk and beak. Seed large, ovoid, yellow and smooth. Vegetative and other floral characters are almost the same as in var. *Erectisiliquosa*, 3-4-valved.

Brassica napus (*Tori, Toria, Lai, Dain, Laita, Sada rayee, Shurshi, Sanchi, Kale sarson*)

Largely grown in the Punjab, Bengal, Assam and the submontane tracts of the United Provinces. Plants shortest amongst the Indian mustards and almost self-sterile. Height 40-115 cm. Tap root slender and tapering. Stem, glaucous, green, glabrous or hairy, with or without purplish tinge in the upper part. Branching usually from the base. Branches shorter than the main stem. Stem-leaves sinnuate lyrate, dark green, generally glaucescent, glabrous or with few hairs on the veins in some cases; 33-13 cm. long and 13-6 cm. wide; auricles rounded and extending half-way round the stem, margins broadly dentate; midrib white and grooved above. Leaves on floral shoots glaucescent, glabrous, triangular-lanceolate, stem-clasping, 14-9 cm. long and 7-2 cm. wide, margins entire. Inflorescence corymb, elongating into a raceme. Pedicel usually glabrous or hairy in those cases where stems bear scattered recurved hairs. Sepals green and sub-erect when young and pale lemon-yellow and hooded. Petals clawed, lemon-yellow. Stamens flush with or overtopping the stigma. Style stout and green. Stigma naphthalene-yellow. Fruit 2-valved, cylindrical and beaded or flat and almost parallel to the axis, 11-4 cm. long. Seed smaller than that of *sarson*, dark brown, rugose; hilum not distinct.

V. Distinguishing characters of the types of *Brassica juncea* and *Brassica campestris**

1. Brassica juncea

Type 1.—Tall, late, robust; branching from the base; branches few; leaves large, very hairy, light green, crumpled, with terminal and lateral uppermost lobes turned inwards at the points of incision; flowers large; sepals light green; petals broad and lemon-yellow, pods spreading; seed dark brown and rugose.

* Plants 75-130-cm. high are termed short, 130-160 cm. high intermediate, and over 160 cm. tall. Plants maturing within 100-130 days are termed early, 130140 days intermediate, and over 140 days late.

Type 2.—Tall and intermediate in maturity; branching above the base; leaves smaller and less hairy than in Type 1, dark green, not crumpled, with uppermost incisions deep; flowers small; sepals light green; petals lemon-yellow, pods spreading; seed black and rugose.

Type 3.—Intermediate in height and maturity; branching above the base; leaves less hairy and smaller than in Type 2, elongated, dark green, not crumpled, with uppermost incisions deep; flowers small; sepals light green; petals lemon-yellow; pods spreading; seed dark brown and rugose.

Type 4.—Intermediate in height but early; branching above the base; leaves less hairy and smaller than in Type 2, elongated, dark green, not crumpled, with uppermost incisions deep; flowers small; sepals light green; petals lemon-yellow; pods spreading; seed dark brown, rugose.

Type 5.—Delicate; very short, very early; branching above the base; leaves small, hairy, with incisions deep; flowers very small, sepals light green; petals lemon-yellow; pods spreading; seed dark brown, rugose.

Type 6.—Short, early, delicate, purplish; branching above the base; leaves small, hairy, with margins dark purplish and folded inwards; flowers very small; sepals light green; petals pale lemon-yellow; pods spreading; seed dark brown, rugose.

Type 7.—Short, early, purplish, delicate, hairy; branching from the base, branches many and shorter than the main stem, bearing both radical and cauline leaves; radical leaves forming a cluster at the base, very hairy, deeply many-lobed; midrib and stalk purple; cauline leaves very hairy, deeply many-lobed; leaves on the floral axis short, narrow, elongated, with margins entire or minutely serrated; flowers small, sepals purplish, petals picric-yellow, small, narrow and veins at the base reddish; pod cylindrical, beaded and not much spreading; seed medium, ovoid, dull brown and faintly rugose.

Type 8.—Short, early; branching above the base; pods semi-appressed; other characters as in Type 3.

Type 9—Tall, late, robust; branching above the base; leaves large, glabrous, with upper incisions shallow; flowers large; sepals light green; petals broad and lemon-yellow; pods spreading; seed medium, brown, very rugose.

Type 10.—Intermediate in height and maturity; vegetative characters as in Type 9; flowers small, sepals light green; petals broad and lemon-yellow; seed medium, brown, very rugose.

Type 11.—Intermediate in height but early. Vegetative and floral characters as in Type 9.

2. *Brassica campestris* L.*

Type 1.—Tall, late, robust, branching from the base and early ; leaves large, hairy, crumpled, dark green and shining, midrib white, terminal lobe rounded, lamina portion connecting terminal lobe and uppermost lateral lobes folded inwards ; flowers large ; petals pale lemon-yellow ; pods spreading, ascending, flat, 2-valved, long, broad, smooth ; seed smooth, yellow, bold.

Type 2.—Tall, late, robust, branching from the base, and about the time of formation of flower buds ; leaves large, hairy, dark green and dull, expanded, midrib white, terminal lobe oblong, lamina portion connecting the terminal and uppermost lateral lobes broad and flat ; flowers large ; petals lemon-yellow ; pods spreading, ascending, flat, 2-valved, long, broad, smooth ; seed smooth, yellow, bold.

Type 3.—Tall, intermediate in maturity, branching from the base, leaves large, hairy, dark green and dull, fully expanded, midrib purplish, terminal lobe ovate, lamina portion connecting the terminal and uppermost lateral lobes somewhat folded inwards ; flowers large ; petals lemon-yellow ; pods spreading, ascending, flat, 2-valved, long, broad, smooth ; seed smooth, yellow, bold.

Type 4.—Tall and intermediate in maturity, branching from the base ; leaves small, hairy, dark green and dull, expanded, midrib white, terminal lobe ovate or oblong, lamina portion connecting the terminal lobe and uppermost lateral lobes broad and flat ; flowers large ; petals lemon-yellow ; pods spreading, ascending, flat, 2-valved, long, broad, smooth ; seed smooth, yellow, bold.

Type 5.—Intermediate in height, late, robust, bushy, branching from the base and early ; leaves large, hairy, somewhat crumpled, dark green and dull, midrib white, terminal lobe ovate, lamina portion connecting the terminal lobe and uppermost lateral lobes irregular and flat, flowers large ; petals lemon-yellow ; pods spreading, ascending, flat, 2-valved, long, broad, smooth ; seed smooth, yellow, bold.

Type 6.—Intermediate in height, late, robust, branching from the base; leaves large, hairy, dark green and dull, somewhat crumpled, midrib white, terminal lobe ovate, lamina portion connecting the terminal lobe and uppermost lateral lobes irregular ; flowers large, petals lemon-yellow ; pods spreading, ascending, flat, 2-valved, long, broad, beaded, beaks long ; seed smooth, yellow, bold.

Type 7.—Intermediate in height and maturity, robust ; branching from the base ; leaves small, hairy, dark green and dull, expanded, midrib white, terminal lobe ovate, lamina portion connecting the terminal lobe and uppermost lateral lobes very narrow and flat ; flowers small ; petals lemon-yellow ; pods spreading, ascending, flat, 2-valved, long broad, smooth ; seed smooth, yellow, bold.

*Plants 75-120 cm. high are termed short, 120-150 cm. high intermediate and 150-175 cm. tall. Plants maturing within 100-130 days are termed early, 130-140 days intermediate, and 140-150 days late.

Type 8.—Intermediate in height and maturity, stem purplish ; branching from the base, main shoot and branches flowering simultaneously ; leaves deep green, hairy, dull and expanded, terminal lobe triangular, lamina portion connecting the terminal lobe and uppermost lateral lobes broad and flat ; flowers large ; petals lemon-yellow, pods spreading, ascending, flat, 2-valved, long, broad smooth ; seed smooth, yellow, bold.

Type 9.—Short, early, stem thin, delicate and purplish ; branching from the base ; main shoot and branches flowering simultaneously ; leaves deep green, dull and expanded, terminal lobe elongated and ovate, lamina portion connecting the terminal lobe and uppermost lateral lobes broad and flat ; flowers small ; petals picric-yellow ; pods spreading, ascending, flat, 2-valved, short, narrow, smooth, and not much spreading ; seed smooth, yellow, bold.

Type 10.—Tall, late, robust ; branching late and about the time of formation of flower buds ; leaves large, glabrous, dark green, dull and expanded, midrib white, terminal lobe oblong, lamina portion connecting the terminal lobe and uppermost lateral lobes broad and flat ; flowers large ; petals lemon-yellow ; pods spreading, ascending, flat, 2-valved, broad, smooth ; seed smooth, yellow, bold.

Type 11.—Intermediate in height and maturity, branching late and about the time of formation of flower buds ; leaves medium in size, glabrous, deep green, dull and expanded, midrib purplish, terimnal lobe triangular or ovate and elongated, lamina connecting the terminal and the uppermost lateral lobes broad and flat ; flowers large, petals lemon-yellow ; pods spreading, ascending, flat, 2-valved, long, broad, smooth ; seed smooth, yellow, bold.

Type 12.—Intermediate in height, early ; other characters as in Type 11.

Type 13.—Intermediate in height, late ; leaves large glabrous, deeply lobed, succulant, emerald green and glossy, terminal lobe large, ovate to rounded ; flowers large ; petals lemon-yellow ; pods spreading, ascending, flat, 2-valved, spreading, long, narrow, smooth ; seed smooth, yellow, bold.

Type 14.—Short, early, glabrous ; stem thin, delicate, purplish ; branching from the base, main shoot and branches flowering simultaneously ; leaves small, glabrous, green, dull, and expanded, terminal lobe elongated and triangular, lamina portion connecting the terminal lobe and uppermost lateral lobes broad and flat ; flowers large ; petals lemon-yellow ; pods spreading, ascending, flat, 2-valved, long, broad, smooth, inflated when mature ; seed smooth, yellow, bold.

Type 15.—Intermediate in height and maturity, hairy ; stem stout and purplish ; branching from the base and late ; leaves large, deeply lobed, deep green and dull, midrib purplish, terminal lobe broadly ovate ; flowers large, petals lemon-yellow, pods spreading, ascending, flat, 2-valved, long, beaded, broad ; seed faintly rugose, brown, bold.

Type 16.—Short, intermediate in maturity, glabrous ; stem purplish ; branching above the base, main shoot and branches flowering simultaneously ; leaves dark green and dull, terminal lobe triangular ; flowers large, petals lemon-yellow ; pods spreading, ascending, flat, 2-valved, long, broad, smooth, dull green, semi-appressed ; seed smooth, brown, bold.

Type 17.—Short, early, glabrous ; stem purplish ; leaves entire, emerald green and glossy ; flowers large, petals pale lemon-yellow, pods spreading, ascending, flat, 2-valved, long, broad, smooth ; seed smooth, brown, bold.

Type 18.—Short, intermediate in maturity, glabrous ; lower leaves irregularly lobed ; flowers large, petals lemon-yellow, pods narrow, short, shining, not much spreading, ascending, flat, 2-valved ; seed smooth, brown, bold.

Type 19.—Short, early and glabrous ; stem delicate purplish ; branching above the base, main shoot and branches flowering simultaneously ; leaves runcinate, succulent, deep green and dull, margin entire ; flowers small ; petals naphthalene-yellow, pods spreading, ascending, flat, 2-valved, long, broad, smooth, semi-appressed ; seed faintly rugose, brown, bold.

Type 20.—Intermediate in height, late, robust, glabrous ; stem purplish ; branching from the base but late ; leaves large, slightly succulent, deep green and glossy, irregularly deeply lobed, slightly crumpled, midrib purplish, terminal lobe broadly ovate, lamina portion connecting the terminal lobe and uppermost lateral lobes broad and flat ; flowers small petals naphthalene-yellow, pods spreading, flat, 2-valved, long, very narrow, beaded ; seed faintly rugose, brown, bold.

Type 21.—Intermediate in height, early, glabrous ; leaves large, succulent, pale green and dull ; flowers very large ; petals lemon-yellow ; pods spreading, ascending, cylindrical, 2-valved, long, slightly beaded ; seed small and smooth, yellow.

Type 22.—Intermediate in height, late and hairy ; leaves large, lyrate, light green and glossy, terminal lobe rounded ; flowers large ; petals lemon-yellow ; pods long, not beaded, spreading, ascending, cylindrical, 2-valved ; seed faintly rugose, brown, small.

Type 23.—Intermediate in height and maturity and hairy ; stem purplish, branching from the base, main stem and branches flowering simultaneously ; leaves lyrate, green and dull ; flowers small ; petals lemon-yellow ; pods spreading, ascending, cylindrical, 2-valved, long, slightly beaded ; seed small, faintly rugose, brown.

Type 24.—Intermediate in height and maturity, robust and glabrous ; leaves large, irregularly deeply lobed, light green, terminal lobe elongated and ovate ; other characters as in Type 22.

Type 25.—Intermediate in height, late, very hairy; radical leaves forming clusters, deep purple, bristly hairy; flowering shoot purplish, hairy, scape-like; flowers large; petals pale lemon-yellow; pods spreading, ascending, cylindrical, 2-valved, long, beaded; seed small, smooth, brown.

Type 26.—Intermediate in height, late, robust, purplish, hairy; branching from the base, main shoot and branches flowering simultaneously; leaves succulent, deep green and dull, midrib purplish, terminal lobe oblong-ovate; flowers large; petals lemon-yellow; pods spreading, ascending, 3-4-valved, long, inflated; beaks long; seed faintly rugose, yellow, bold.

Type 27.—Intermediate in height and maturity, robust, purplish, hairy; branching from the base; leaves large, oblong-ovate, deep green and dull, midrib purplish; flowers large; petals pale lemon-yellow; pods with short stalks, slightly inflated, spreading; ascending, 3-4-valved; seed smooth, yellow, bold.

Type 28.—Pods with long stalks, very much inflated; other characters as in Type 27.

Type 29.—Tall, intermediate in maturity, purplish, glabrous; branching from the base; leaves small, green and dull, terminal lobe ovate; flowers large; petals pale lemon-yellow; pods spreading, ascending, 3-4-valved, long, slightly inflated; beaks long; seed smooth, yellow, bold.

Type 30.—Intermediate in height and maturity, purplish, glabrous; branching above the base, main stem and branches flowering simultaneously; leaves small, light green and dull, terminal lobe ovate; flowers large; petals lemon-yellow; pods spreading, ascending, 3-4-valved, long, much inflated; beaks long; seed smooth, yellow, bold.

Type 31.—Intermediate in height and maturity; pods very much inflated; beaks short; other characters as in Type 29.

Type 32.—Short, early, robust, purplish, glabrous; branching from the base and early; leaves large, green and dull, terminal lobe oblong-ovate; petals naph thalene-yellow; flowers small; pods long, spreading, ascending, 3-4-valved, inflated; beaks long; seed smooth, yellow, bold.

Type 33.—Short, glabrous, early; leaves deep green and dull; flowers large; petals lemon-yellow; pods long, pendant, flat, 2-valved, broad, smooth; seed smooth, yellow, bold.

Type 34.—Tall, late, robust, purplish and hairy, branching from the base and early; leaves large, dark green and dull, terminal lobe rounded; flowers large; petals lemon-yellow; pods very long, pendant, 3-4-valved, inflated and with long stalk; seed smooth, yellow, bold.

Type 35.—Short, early, robust, purple, glabrous, branching above the base and late; leaves large, green and dull, terminal lobe oblong-ovate; flowers large, petals pale lemon-yellow; pods long, pendant, 3-4-valved, inflated, beaded; beaks long; seed smooth, yellow, bold.

VI. Summary

1. A general plan of the classification of cultivated Indian mustards, belonging to *Brassica nigra* Koch, *Brassica rugosa* Prain, *Brassica juncea* H. f. & T., *Brassica campestris* L. and *Brassica napus* L. is presented.

2. A key to the mustards is worked out.

3. The species and their varieties are described and the distinguishing characters of the types of *Brassica juncea* and *Brassica campestris* are given.

4. A few observations on the method of sowing, flowering and pollination are recorded in Appendix I.

5. The localities from where the original seed samples were obtained are noted in Appendix II.

VII. REFERENCES

Akhtar, A. R. (1932). *Ind. J. Agric. Sci.* **2**, 280-292.

Cooke, T. (1903). 'Flora of the Bombay Presidency', Vol. I, pp. 34, 35.

Dobbs, A. C. (1924). *Agric. J. Ind.* **19**, 196.

Duthie, J. F. and Fuller, J. B. (1882). 'Field and Garden Crops', part 2, pp. 28-34.

Duthie, J. F. (1903). 'Flora of the Upper Gangetic Plain', Vol. I, pp. 155-157.

Hooker, J. D. (1872). Flora of British India, Vol. I, pp. 155-157.

Howard, A., Howard, G.L. C. and Khan, A. R. (1910). *Mem. Dept. Agric. Ind., Bot. Ser.* **3**, No. 6, pp. 317-320.

Howard, A., Howard, G.L. C. and Khan, A. R. (1915). *Mem. Dept. Agric. Ind., Bot. Ser.* **7**, No. 7.

Prain, D. (1898). *Agric. Ledger* **1**.

Schulz, O. E. (1919). 'Cruciferae—Brassiceae, das Pflanzenreich', IV, 105.

Viehoever, A., Clevenger, J. F. and Ewing, G. O. (1921). *J. Agric. Res.* **20**, 117-140.

Report on the operations of the Dept. Agric. Punjab, Part I, p. 20, 1931.

VIII. Appendix I

Observations on the method of sowing, flowering and pollination

The biology of the mustards has been adequately described by Howard, Howard and Khan [1915] and observations in this paper are confined to a few additional remarks which may be useful to scientific workers on this crop.

Mustards, especially *Brassica nigra*, do not tolerate deep sowing. When sown in deep furrows made by the plough, they do not germinate uniformly. The best results are obtained by sowing the seeds in shallow furrows 1-2 inches deep. Germination in this case is very uniform and quick.

The time of flowering and maturity varies in different mustards. Though they were all sown on the same date (16th October), the *tori* and some of the early types of *rai* were the first to mature. The *tori* plants began to flower from the 14th November when they were only about 9 inches in height and became ready for harvesting from the 1st February. Some of the types of *rai* (Types 5, 6 and 8) ripened early and were harvested about the same time as *tori*. The *sarson* varieties were next to mature. They commenced flowering from the 25th November and some of the early types (Nos. 9, 14, 19, 21, 32, 35) were ready for harvesting about the 7th February. The late types *rai* and *sarson* took about 140-150 days to complete their growth. *Brassica rugosa* was the last to mature amongst the mustards. These plants flowered about the same time as late *rai* and *sarson* but were not ready for harvest before the end of March.

A number of plants belonging to *B. nigra* (Benarasi *rai*), *B. rugosa* (Pahadi *rai*) and *B. juncea* (*Rai*), *B. compestris* (*sarson*), *B. napus* (*Tori*) were bagged to obtain self-pollinated seeds and also to study the extent of natural cross-pollination in these plants. In *tori* and Benarasi *rai* only a few seeds were obtained from each of the bagged plants which shows that these mustards are almost self-sterile and natural cross-pollination predominates in them. On the other hand the bagged plants of *sarson* and *rai* form seeds freely and appear to be naturally self-pollinated. A certain amount of cross-pollination would, however, be expected in these mustards through the bees which visit the flowers in large numbers.

IX. Appendix II*

Places from where the original seed samples were obtained

1. Brassica nigra

North-West Frontier Province :—Peshawar, Tarujabba 365, 366. Punjab :—Lyallpur 546. United Provinces :—Etawah 544 ; Moradabad, Bilari 550 ; Jaunpur 551 ; Pilibhit 359, 360 ; Atraula 543 ; Bareilly 545 ; Mirzapur 352, 353, 374. Madras :—Bezwada, Krishna-Lankas 547 ; Krishna, Chalapalli 548 ; Ganjam, Berhampur 549.

2. Brassica rugosa

United Provinces :—Kumaon hills, Almora 716, 717, 718.

3. Brassica juncea

Type 1.—Punjab :—Lyallpur 518, 519. United Provinces :—Sitapur 493, 494 ; Mirzapur 496, 497, 554 ; Aligarh 495, 553 ; Bahraich 506, 508 ; Allahabad 515, 542 ; Etawah 505 ; Rura 514 ; Bengal :—Maida 531 ; Dacca 534, 575. Bombay :—Gujrat, Dao (Dolka) 507. Madras :—Ganjam, Berhampur 520.

* The numbers refer to the herbarium sheets and the names of places to the sources from which the original seed samples were obtained.

Type 2.—United Provinces :—Benares 510, 511 ; Fyzabad 512, 513 ; Bahraich 504 ; Kulpahad 523 ; Mainpuri 529, 530. Bihar and Orissa :—Sabour 524. Bengal :—Tipperah 536.

Type 3.—United Provinces :—Partabgarh—Darwa market 498, 499 ; Maholi 500, 501 ; Gonda 502, 503. Bihar and Orissa :—Sabour 525 ; Patna 537.

Type 4.—Bihar and Orissa :—Sabour 526. Bengal :—Dacca 534, 575 ; Barisal 516, 517. Madras :—Ganjam, Aska 521.

Type 5.—Bengal :—Rangmati 535 ; Twenty-four Parganas, Barasat 540, 541.

Type 6.—Bengal :—Chittagong, Ramu 538, 539.

Type 7.—Punjab :—Lyallpur 719, 720, 724.

Type 8.—Bengal :—Rangpur 533.

Type 9.—Punjab :—Lyallpur 568, 569. United Provinces :—Benares 563 ; Fyzabad 564, 565 ; Aligarh 578 ; Rura 567. Bengal :—Maida 574.

Type 10.—United Provinces :—Partabgarh, Darwa market 555, 556, 558. Madras :—Ganjam, Berhampur 570, 571.

Type 11.—United Provinces :—Partabgarh 552 ; Maholi 559, 560, 561, 562 ; Fyzabad 566 ; Jaunpur 577. Bihar and Orissa :—Patna 576. Madras :—Ganjam, Aska 572, 573.

4. Brassica campestris

Type 1.—United Provinces :—Aligarh 582, 583.

Type 2.—United Provinces :—Rura 599, 600.

Type 3.—Punjab :—Lyallpur 615, 616.

Type 4.—United Provinces :—Atarra 603 ; Kulpahad 619 ; Hardoi 626, 627, 628, 629.

Type 5.—United Provinces :—Etawah 593, 594.

Type 6.—United Provinces :—Cawnpore 601.

Type 7.—Bombay :—Ahmadabad, Paranty 609, 610.

Type 8.—United Provinces :—Mirzapur 584, 585 ; Allahabad 605, 606. Bengal :—Barisal 611, 612.

Type 9.—Bengal :—Birbhum 638.

Type 10.—United Provinces :—Mainpuri 630 ; Cawnpore 602 ; Atarra 604.

Type 11.—United Provinces :—Kulpahad 726 ; Atraula 586, 587.

Type 12.—United Provinces :—Gonda 588, 589.

Type 13.—United Provinces :—Bareilly 647.

Type 14.—United Provinces :—Bijnor 640. Bihar and Orissa :—Patna 642, 643. Bengal :—634, 635, Birbhum 637, 639.

Type 15.—United Provinces :—Sitapur 713.

Type 16.—United Provinces :—Benares 598 ; Jaunpur 644.

Type 17.—United Provinces :—Partabgarh, Maholi 645.

Type 18.—United Provinces :—Benares 597.

Type 19.—United Provinces :—Partabgarh, Maholi 646.

Type 20.—United Provinces :—Partabgarh, Maholi 648 ; Kulpahad 725. Bihar and Orissa :—Ranga 625.

Type 21.—United Provinces :—Etawah 592.

Type 22.—United Provinces :—Rura 721. Punjab :—Lyallpur 617.

Type 23.—United Provinces :—Atarra 650.

Type 24.—Punjab :—Lyallpur 618.

Type 25.—North-West Frontier Province :—Peshawar, Tarujabba 489, 490, 491, 492, 715.

Type 26.—United Provinces :—Sitapur 714.

Type 27.—United Provinces :—Sitapur 579 ; Lakhimpur 580 ; Bareilly 613, 614. Bihar and Orissa :—Sabour 621.

Type 28.—United Provinces :—Bareilly 722.

Type 29.—United Provinces :—Lakhimpur 581.

Type 30.—United Provinces :—Pilibhit 607, 608.

Type 31.—United Provinces :—Bahraich 590, 591.

Type 32.—Bihar and Orissa :—Sabour 621.

Type 33.*—Bengal :—Calcutta, Sibpur Experimental Farm, Cultivated (Prain 481).

Type 34.—United Provinces :—Lakhimpur, Gola Gokarannath 595, 596.

Type 35.—Bihar and Orissa :—Ranga 623.

5. *Brassica napus*

Punjab :—Lyallpur 723. United Provinces :—Sitapur 653, 654, 655, 706 ; Mirzapur 50, 182, 183, 184, 676 ; Kulpahad 686, 687, 688; Atarra 87, 88, 89, 90 ; Jaunpur 80 ; Lakhimpur, Gola Gokarannath 79, 682, 710 ; Gonda 76, 185, 186, 679 ; Atraula 122, 181, 677, 678 ; Bahraich 70, 71, 680, 681 ; Nanpara 82, 84, 85, 86, 683 ; Partabgarh 17. Bihar and Orissa :—Ranga 100, 101, 102, 103, 104, 689. Bengal :—Dacca 150, 151, 152, 698, 700 ; Dinajpur 155, 156, 157, 691, 693, 694 ; Rangamati 114, 115, 116, 117 ; Maida 105, 106, 109, 690 ; Birbhum 161, 162, 701, 702 ; Twenty-four Parganas, Basirhat 163, 164, 165 ; Tipperah 168, 169, 170, 703 ; Krishnanagar (Nadia) 173, 174, 175, 704 ; Chittagong, Ramu 176, 177, 178, 179, 180 ; Barisal 705. Assam :—Sylhet 1, 13, 27, 35, 43, 62, 670, 671, 672, 707, 708, 709 ; Gauhati 51, 52, 53, 54, 55, 56, 675 ; Sibsagar 57, 58, 59, 60, 61, 673, 674. Madras :—Ganjam, Aska 95, 96, 97, 98, 99, 685 ; Berhampur 684.

---

*Based on the herbarium specimens from the Royal Botanical Gardens, Calcutta.

SOME SOIL-HETEROGENEITY TRIALS AT PUSA AND THE SIZE AND SHAPE OF EXPERIMENTAL PLOTS

BY

R. D. BOSE, B.Sc.,

Offg. Assistant Economic Botanist, Pusa

(Received for publication on 3rd May 1935)

(With four text-figures)

I. SOIL-HETEROGENEITY TRIALS

In theory, field trials should be conducted under uniform conditions of soil and culture. This, however, is an unattainable ideal since an absolutely uniform piece of land hardly exists in nature and therefore methods must be adopted which lessen or eliminate the effects of soil-heterogeneity. Fields which from inspection appeared satisfactorily uniform have been shown by Harris [1915] and many others to be very heterogeneous in some cases, and the extent to which even a small field can show variations in fertility is demonstrated by the present results of uniformity trials conducted at Pusa.

A field about one-fourth of an acre in area was sown in three consecutive years with Pusa barley Type 21, Pusa 52 wheat and Pusa lentil Type 11, respectively. At harvest a substantial border was removed from all sides and the remaining field was sub-divided into 390 ultimate plots, each four feet square. The produce from these ultimate units was harvested and threshed separately. Combinations of 2×3 such ultimate plots were made for the purpose of drawing contour maps of the field based on the yields, the field consisting of 65 such combination plots, 5 plots running from west to east by 13 plots running north to south as shown in Fig. 1. Assuming that the average yield of each plot is located at the centre of a plot, the points at which the yields were 10, 20, 30, 40, etc., per cent above or below the mean yield of the crop were marked on the field plan. These points were joined and the contour maps shown in the figure given below were constructed.

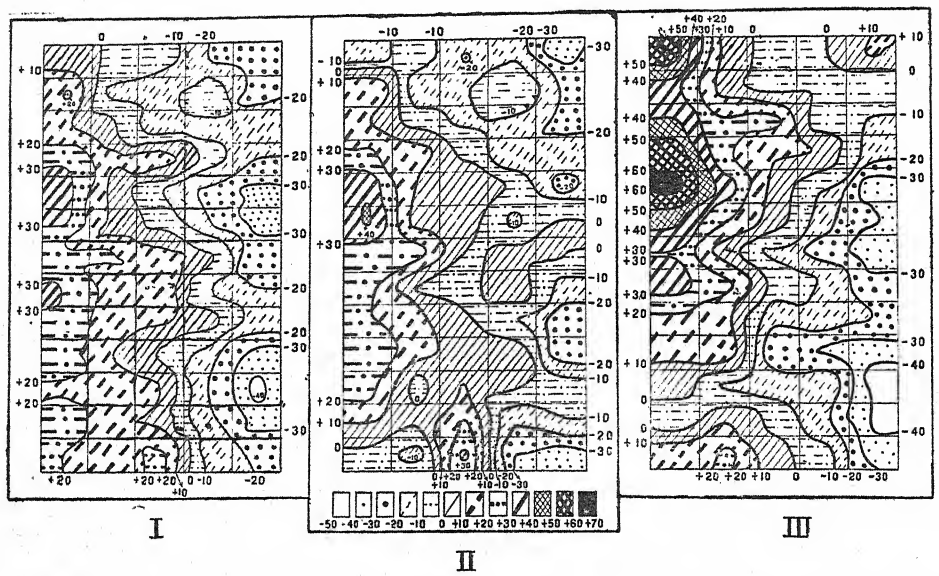


Fig. 1. Contour maps of the field based on yields of (*i*) barley, (*ii*) wheat and (*iii*) lentils, obtained in three successive years from the same experimental field.

It is evident that the yield varied greatly between different sub-plots in the same field, and that this heterogeneity was systematic to a considerable extent is also apparent as the fertility contour lines run, to a very pronounced degree, more or less parallel to the direction of the columns running north to south. The contour lines show a good deal of similarity in all the three maps shown above. The differences present are due, of course, to the variability in the yielding power of the different crops under consideration and its relation to the mean yield of the crop under the conditions of the experiment.

Generally speaking, soil-heterogeneity may exist either as a gradual change of productivity from one side of the field to the other, corresponding to the line of slope or the pathway of irrigation, etc., or as random patches of ground of higher or lower fertility. This 'patchiness' of a field is due to adjacent plots into which the field is divided resembling each other, and represents the most common type of soil heterogeneity. These irregularities in the field may be so powerful sometimes as to vitiate the results of varietal or breeding trials, giving significance to yields in situations where it is not actually present.

It must be remembered that the main object of the modern methods of field trials, and the application of refined methods of analysis to their results is to allow, as far as possible, for this effect of soil-heterogeneity. Although Fisher [1932] does not consider it necessary to crop the experimental plots in the year previous to the experiment, as it involves double the labour of the experiment and a year's delay

before the result is made available, it is profitable at times if the experimenter has some measure of the nature of his field and knows in which direction the soil-fertility varies the most. He has then the advantage of having valuable information to assist him in deciding on the layout of the experiment and on the correct size and shape of plots which he should choose. It is also possible for him then to discard fields of 'patchy' fertility for comparative trials. In agricultural experimental and demonstration stations where the testing of improved varieties of crops or their response to different manurial or other treatments forms an important item of work, a knowledge of the heterogeneity of different fields proves very advantageous indeed.

Harris [1915] has suggested that soil-heterogeneity may be measured by a coefficient which shows the degree of correlation between the yields of contiguous plots. Fisher's analysis of variance may also be employed to determine the drift in the fertility of the field. Whereas Harris' method provides a measure of heterogeneity present in the whole field, Fisher's analysis of variance method not only gives this measure but also clearly sets forth the direction of the fertility gradient and should, therefore, prove a more comprehensive method for such work. It is the purpose of this part of the paper to present the results of the heterogeinty tests conducted at Pusa and to give full arithmetical details of the methods of calculations that were employed.

Historical

Mercer and Hall [1911] harvested 200 plots of mangolds, each 1/200th of an acre in size and 500 wheat plots, each 1/500th of an acre, and calculated the probable errors for combinations of different sizes and number of replications. They found that the experimental error diminished with the size of the plot but the reduction was small when the plots were above 1/40th of an acre. These authors reported that their mangolds 'looked a uniform and fairly heavy crop for the season and soil' while for their wheat 'a very uniform area was selected'. Harris used the same data for calculating the coefficient of correlation as a measure of soil-heterogeneity and found it to be—

For mangolds for a 2×2 combination :—

$r = +0 \cdot 346 \pm 0 \cdot 042$ (for roots)

$r = +0 \cdot 466 \pm 0 \cdot 037$ (for leaves)

For wheat for a 4×5 combination :—

$r = +0 \cdot 186 \pm 0 \cdot 029$ (for grain)

$r = +0 \cdot 343 \pm 0 \cdot 027$ (for straw)

Montgomery [1912] harvested 500 small plots of wheat separately from a field which was 'of about average uniformity and fertility' and tested the nitrogen content of the individual plots with a view to forming a basis of the heterogeneity

in the soil. Analysing this data Harris obtained a coefficient of correlation of $0\cdot603\pm0\cdot029$ for grain and $0\cdot115\pm0\cdot044$ for percentage of nitrogen for a 2×2 grouping. Lehmann [1901-1909] has given a series of data derived from the yields of grain and straw of *ragi* (*Eleusine coracana* Gaertn.) cultivated at the Hebbal Farm near Bangalore in India and these were analysed by Harris [1920] for correlation between the yield of grain, of straw, and of grain and straw. The following figures were obtained :—

——	1905	1906	1907
Grain	$0\cdot735\pm\cdot031$	$0\cdot138\pm\cdot065$	$0\cdot716\pm\cdot032$
Straw	$0\cdot424\pm\cdot055$	$0\cdot164\pm\cdot065$	$0\cdot573\pm\cdot045$
Total yield	$0\cdot415\pm\cdot055$	$0\cdot145\pm\cdot065$	$0\cdot636\pm\cdot040$

' The practical conclusion to be drawn from this result is that an experimental field which might be demonstrated to be sensibly uniform for one crop plant or for one season might not prove to be so for another crop or in a different season ' [Harris, 1920].

Parnell [1919] in Madras determined the amount of soil variability in the typical paddy soils of the Presidency and found that they varied from 7·7 per cent to 13·9 per cent for the probable error of differences between two plots and for small plots, he came to the conclusion, based on a number of cases, that an area of 50×4 feet repeated 8 times would reduce the probable error of mean difference to about 2 per cent. At the Maruteru Paddy Breeding Station in Madras experiments of a preliminary type of the nature of " blank-tests " were conducted by Iliffe and Srinivasan [1926] to estimate the amount of error with a view to choosing the correct size and shape of plots. It was found that a 40 feet$\times$5 feet plot, replicated six times, reduced the probable error of difference to such an extent that a 10 per cent difference in the experiments would be significant.

Garber [1926] experimented with 270 plots of 68 feet$\times$2 feet for two years with wheat and oats and found that the coefficients of correlation for oats ranged rom $0\cdot542\pm0\cdot030$ to $0\cdot694\pm0\cdot031$ and for wheat from $0\cdot516\pm0\cdot031$ to $0\cdot617\pm0\cdot037$ and that there was some tendency for the plots which produced relatively high yields of oats to produce relatively high yields of wheat in the following year. There was also a correlation of $0\cdot364\pm0\cdot036$ between the yields of the same plots of the two crops in the two successive years.

Sanders [1930] has suggested the application of the analysis of covariance in order to increase the precision of experimental results on the basis of a knowledge of the yielding capacities of the same plots in a previous or preliminary trial. He tried to determine whether soil variations were sufficiently constant from year to

year to give useful corrections in the yields of experimental plots by considering the yields of the same plots under previous uniformity trials. Considering the published results of uniformity trials with cereals carried out on two fields at Aarslev (Denmark) during the years 1906 to 1911, he found that while in one field the precision of the experiment was increased by 150 per cent by utilising the previous records and correction of yields by the application of the method of covariance, yet in another field the plots showed no constancy in yield. Eden [1931], however, obtained results of increased precision with a perennial crop, tea, by correcting experimental yields on the basis of previous cropping.

Material and method

An area measuring 0·24 acre, which was selected for running a yield trial experiment with some Pusa barleys in 1928-29 [Shaw and Bose, 1929], was chosen for conducting this trial. The soil consists of a sandy loam and no manure nor any irrigation was applied to this plot at any stage of the experiment. The following crops were grown :—

Winter of	1927-28	Lucerne.
,,	1928-29	Barley yield-trials [Shaw and Bose, 1929].
,,	1929-30	Heterogeneity test with Pusa barley Type 21 [Bose, 1931].
,,	1930-31	Heterogeneity test with wheat Pusa 52 [Howard, 1927].
,,	1931-32	Heterogeneity trial with Pusa lentil Type 11 [Shaw and Bose, 1928].

All the crops used in this experiment were crops which are grown in India in the cold season from October to March. Sowing therefore took place about the end of October and harvest during the second half of March. During the period April to October each year the field was left fallow. Usual cultivation and weeding were given. For the heterogeneity or ' uniformity ' trials the crop was drilled in rows one foot apart, the usual* seed rate for the particular crop was always employed. At harvest borders of three feet each on the east and west and six feet each on the north and south of the field were removed and the remaining central area was divided into 390 equal-sized small plots, each four feet square. There were in all fifteen of these ultimate plots running east to west and 26 ultimate plots going north to south making in all 390 small plots (Fig. 2). The produce from each ultimate plot was threshed by hand and weighed separately. Care was taken to see that all weighings were done after the grain had been sun-dried for a number of days so as to obtain an approximately uniform moisture content.

---

* Wheat 70 lb. per acre.
Barley 70 lb. per acre.
Lentils 16 lb. per acre.

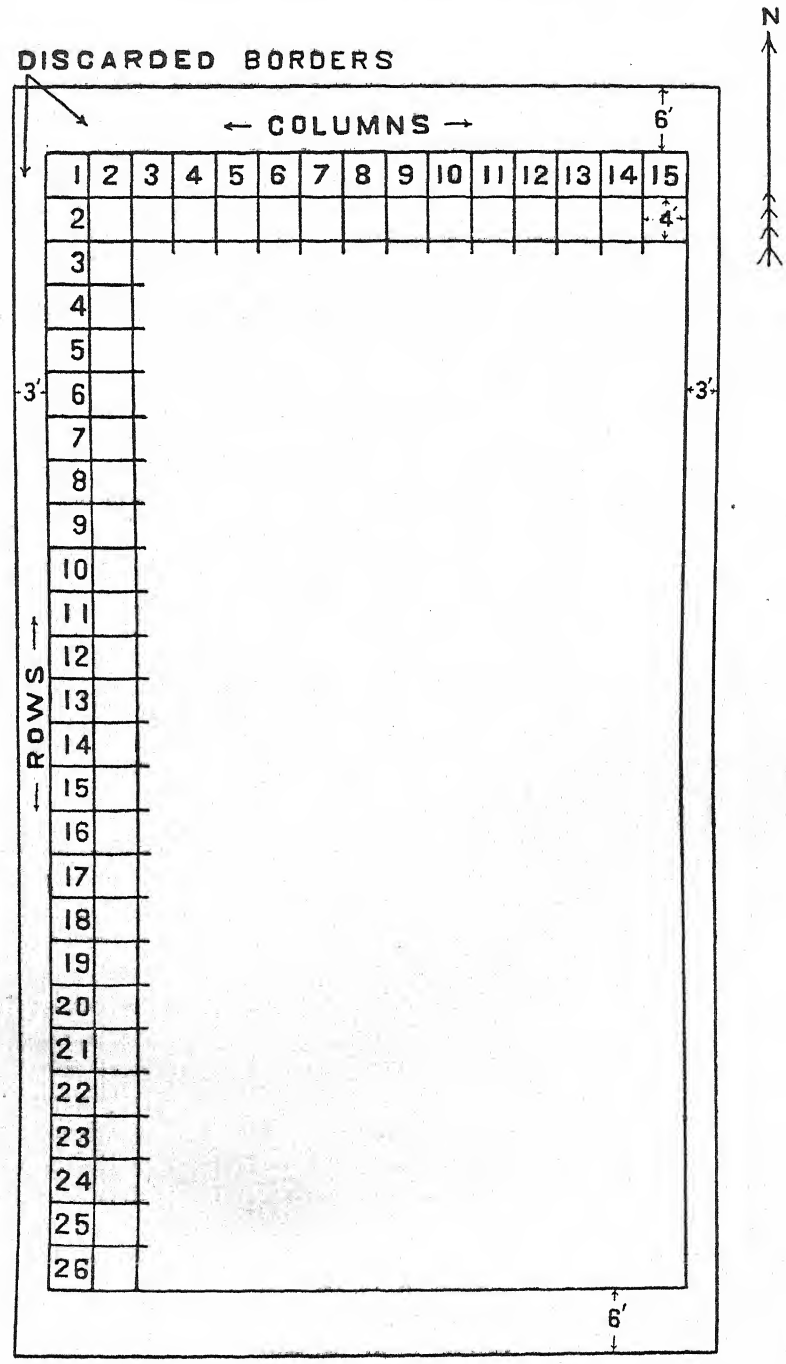


Fig. 2. Diagrammatic representation of the division of the experimental plot into ultimate units just before harvest.

Harris' method [1915] of estimating soil-heterogeneity based on the determination of the coefficient of correlation between the yielding powers of adjacent plots was employed in these studies and the results were further analysed by Fisher's analysis of variance which appears to be an excellent weapon for attacking such investigations.

Experimental results

(1) Harris' method

The yields of ultimate plots of barley obtained in 1929-30 are shown in Table I.

Table I

Yield of barley Type 21 from ultimate plots 4 ft. × 4 ft. in area

Row No.	Yield in grms. from column No.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	880	595	835	635	615	645	635	730	610	455	535	525	500	350	310
2	715	515	530	540	720	530	655	480	470	470	490	565	675	630	300
3	970	850	755	525	510	565	575	570	535	690	600	685	600	530	300
4	410	865	760	500	500	735	655	465	380	610	565	625	610	470	365
5	585	740	810	680	510	675	510	580	280	430	490	670	620	600	390
6	630	685	820	775	570	665	610	705	480	580	525	555	670	510	530
7	680	615	930	810	695	635	875	750	540	705	640	490	445	595	560
8	855	970	830	765	775	665	740	900	650	675	615	545	600	355	495
9	1010	635	890	830	585	545	500	615	630	510	475	520	540	420	300
10	890	810	935	745	675	615	495	720	525	495	540	300	595	430	265
11	805	635	850	755	625	525	485	795	470	320	570	460	500	490	315
12	1040	725	910	765	710	660	525	750	700	610	580	565	545	510	180
13	570	810	870	900	785	705	650	745	710	790	580	580	560	585	395
14	790	635	960	885	715	630	710	955	730	685	670	625	530	465	405
15	920	750	890	765	745	595	515	1030	785	670	440	375	450	500	505
16	895	770	870	720	840	720	780	805	650	610	610	800	555	500	585
17	810	775	800	625	865	785	770	630	630	785	705	565	610	660	490
18	935	675	830	750	635	760	790	720	700	790	525	605	690	360	460
19	900	720	870	725	625	665	465	660	690	670	620	570	650	310	335
20	755	600	930	695	815	690	785	650	535	430	460	470	420	435	305
21	670	720	800	670	760	750	610	610	650	415	630	545	425	450	315
22	780	700	810	790	810	705	820	850	675	590	550	350	370	360	280
23	885	740	960	690	755	680	630	635	850	590	490	755	550	480	365
24	940	680	700	825	665	650	615	700	675	460	450	410	420	355	390
25	880	575	630	610	625	695	850	775	730	425	380	605	650	375	280
26	955	765	850	810	725	710	920	730	710	680	600	660	710	420	515

Total sum of squares of all individual yields

$\Sigma p^2 = 166978025$

Mean of ultimate plots or $\bar{p} = \frac{247355}{390} = 634 \cdot 2436$

Two series of groupings hereafter called the 1×5 combination and the 2×5 combination respectively were made up by totalling together the yields of one plot north to south and five plots east to west in the first case, and two plots north to south and five plots east to west in the second case. Thus, out of 390 ultimate plots, 78 combination plots could be made up for 1×5 combination and 39 combination plots formed for the 2×5 combination. The yields of these two series of combination plots are shown in Tables II and III, respectively.

TABLE II

Yield of barley in 1×5 combinaton plots

Row No.	Yield in grms.		
	Block I	Block II	Block III
1	3560	3075	2220
2	3020	2605	2660
3	3610	2935	2715
4	3035	2845	2635
5	3325	2475	2770
6	3480	3040	2790
7	3730	3505	2730
8	4195	3630	2610
9	3950	2800	2255
10	4055	2850	2130
11	3670	2595	2335
12	4150	3245	2380
13	3935	3600	2700
14	3985	3710	2695
15	4070	3595	2270
16	4095	3565	3050
17	3875	3600	3030
18	3825	3760	2640
19	3840	3150	2485
20	3795	3090	2090
21	3620	3035	2365
22	3890	3640	1910
23	4030	3385	2640
24	3810	3100	2025
25	3320	3475	2290
26	4105	3750	2905
Total sum of squares	371810375	+ 275598325	+ 166475925

i.e., $\Sigma Op^2 = 813884625$

TABLE III

Yield of barley in 2×5 combination plots

Row No.	Yield in grms.		
	Block I	Block II	Block III
1 and 2	6580	5680	4880
3 and 4	6645	5780	5350
5 and 6	6805	5515	5560
7 and 8	7925	7135	5340
9 and 10	8005	5650	4385
11 and 12	7820	5840	4715
13 and 14	7920	7310	5395
15 and 16	8165	7160	5320
17 and 18	7700	7360	5670
19 and 20	7635	6240	4575
21 and 22	7510	6675	4275
23 and 24	7840	6485	4665
25 and 26	7425	7225	5195
Total sum of squares	741771675 +	549642725 +	330839375

i.e., $\Sigma Cp^2 = 1622253775$

As already mentioned, the coefficient of correlation is used as an index of soil uniformity as advanced by Harris [1915]. This constant usually termed $r_{p_1p_2}$ is calculated according to the following formula :—

$$r_{p_1p_2} = \frac{[\{\Sigma(Cp^2) - \Sigma(p^2)\}/m\{n(n-1)\}] - \bar{p}^2}{\sigma p^2}$$

where

$r_{p_1p_2}$ = the coefficient of correlation sought.

$\Sigma(Cp^2)$ = sum of squares of group yields of combination plots.

$\Sigma(p^2)$ = sum of squares of the yields of ultimate units.

$\bar{p}^2$ = square of the average yield of all ultimate units.

n = number of units in each group.

m = number of groups of combination plots.

σp^2 = square of the standard deviation of yield for the ultimate units.

The squares of all yields, *i.e.*, of ultimate plots or of combination plots are written down and summated, and the standard deviation of yields for the ultimate units is obtained by the formula—

$$\sigma p = \sqrt{\frac{\Sigma(p^2)}{mn} - (\bar{p})^2}$$

where as stated above $\Sigma(p^2)$ is the sum of squares of the yields of the ultimate units and $\bar{p}$ is the average yield of all ultimate units. By substituting the actual values obtained for the barley experiment we get—

For the 1×5 combination

$$r_{p_1p_2} = \frac{[\{813884625-166978025\}/78\{5(5-1)\}]-(634\cdot2436)^2}{\left\{\sqrt{\frac{166978025}{390}-\left(\frac{247355}{390}\right)^2}\right\}^2}$$

$$= \frac{\{646906600/1560\} - 402264\cdot9441}{\{\sqrt{428148\cdot7820}-402264\cdot9441^2}$$

$$= \frac{414683\cdot7179-402264\cdot9441}{25883\cdot8379} = 0\cdot4798$$

$$\text{Standard error} = \pm\frac{(1-r^2)}{\sqrt{n}} = \pm 0\cdot0390$$

$$\therefore\ r_{p_1p_2} = 0\cdot4798 \pm 0\cdot0390$$

For the 2×5 combination

$$r_{p_1p_2} = \frac{[\{1622253775-166978025\}/\ 39\{10(10-1)\}] - (634\cdot2436)^2}{\left\{\sqrt{\frac{166978025}{390}-\left(\frac{247355}{390}\right)^2}\right\}^2}$$

$$= \frac{\{1455275750/3510\}-402264\cdot9441}{25883\cdot8379}$$

$$= \frac{414608\cdot4758-402264\cdot9441}{25883\cdot8379} = 0\cdot4769$$

$$\text{Standard error} = \pm\frac{(1-r^2)}{\sqrt{n}} = \pm 0\cdot0391$$

$$\therefore\ r_{p_1p_2} = 0\cdot4769 \pm 0\cdot0391$$

It may be pointed out that the large figures in these calculations have been obtained only because yields were retained in units of grammes. A calculating machine, the 'Comptometer' having been used, no difficulty whatsoever was experienced in handling these figures. The yields could have been converted into decagrammes or even kilogrammes and the size of figures for their respective squares could thus have been reduced.

Wheat, 1930-31

In the following year Pusa 52 wheat was similarly sown in this very area and Table IV given below shows the yield of ultimate plots.

TABLE IV

Yield of Pusa 52 wheat from ultimate plots 4 ft. × 4 ft. in area

Row No.	Yield in grms. from column No.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	70	220	265	230	248	322	205	250	180	225	235	280	155	190	120
2	248	258	215	220	200	185	185	258	120	215	145	190	210	180	120
3	275	402	225	120	275	195	200	220	217	185	335	250	235	190	170
4	270	400	385	240	225	200	200	252	255	268	235	220	300	130	170
5	195	230	335	272	200	210	190	340	205	222	295	160	230	235	155
6	240	348	335	296	185	250	185	235	160	170	225	235	250	132	140
7	275	280	325	390	200	215	210	175	212	142	270	240	220	120	270
8	218	335	400	370	235	340	260	310	305	160	200	200	335	232	100
9	260	415	430	365	230	220	245	370	232	232	222	215	235	150	130
10	275	375	360	340	240	250	160	375	257	232	340	260	260	190	210
11	335	392	305	300	200	232	225	345	210	280	180	185	310	280	245
12	345	380	360	320	265	255	220	420	200	155	250	255	235	260	200
13	270	310	415	385	250	262	230	290	190	280	320	220	290	160	240
14	155	395	355	398	330	265	255	350	172	260	328	190	280	255	295
15	295	318	305	408	215	247	235	400	182	280	315	245	345	140	250
16	335	185	422	190	220	210	155	335	130	265	245	300	208	140	220
17	242	280	295	305	225	210	295	345	132	258	215	132	130	135	205
18	305	310	400	312	450	210	305	290	182	342	275	145	210	180	255
19	380	250	320	322	300	327	232	290	320	305	260	165	140	140	190
20	318	367	355	225	348	232	230	320	150	345	290	265	265	160	230
21	347	412	280	300	290	205	287	315	185	355	220	240	280	180	295
22	260	308	305	230	230	205	315	385	185	225	257	222	237	200	230
23	264	308	280	270	310	235	265	380	285	227	235	200	267	165	210
24	248	318	280	270	205	275	320	328	245	192	180	200	235	185	240
25	210	315	265	260	155	200	415	370	255	170	150	190	245	245	115
26	230	302	180	270	170	200	335	312	315	260	100	187	155	150	82

Total sum of squares of all individual yields

$$\Sigma p^2 = 26741531$$

Mean of ultimate plots or $\bar{p} = \frac{98217}{390} = 251 \cdot 838$ grms.

Lentils, 1931-32

Similarly in Table V the yield of lentils from ultimate plots obtained from this very field in the following year are shown.

TABLE V

Yield of lentil Type 11 from ultimate plots 4 ft. × 4 ft. in area

Row No.	Yield in grms. from column No.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	412	381	312	182	272	231	255	217	254	166	259	272	188	249	179
2	325	329	265	229	214	178	147	149	178	208	164	205	291	251	278
3	312	300	311	253	234	168	152	208	224	222	181	219	197	176	241
4	288	305	235	276	218	185	259	191	180	140	252	221	148	207	220
5	273	306	272	306	232	217	286	177	148	161	204	228	189	205	184
6	332	337	310	283	280	265	220	279	216	235	174	178	177	187	220
7	431	333	304	328	265	201	249	252	251	250	206	240	186	203	181
8	342	331	313	351	204	218	265	267	174	230	240	140	143	171	189
9	401	315	317	344	259	267	279	227	277	203	241	165	126	149	124
10	394	352	325	338	311	233	268	181	133	178	177	202	158	155	129
11	275	357	300	261	275	271	285	175	176	165	207	163	160	161	135
12	338	299	334	289	218	249	310	163	263	215	181	143	159	125	171
13	285	279	266	257	200	200	266	162	220	188	186	160	109	122	155
14	306	269	272	253	178	252	224	81	169	211	108	127	184	109	139
15	253	260	312	284	265	282	290	143	161	159	179	180	211	160	130
16	276	319	331	232	278	243	239	194	220	253	192	185	126	138	177
17	235	255	266	268	276	217	203	131	181	180	210	140	198	164	164
18	252	260	235	259	203	229	236	137	173	194	109	266	220	122	161
19	305	230	182	234	237	248	190	128	126	148	173	134	216	105	143
20	226	240	259	305	272	212	193	143	141	173	142	125	135	96	97
21	307	255	186	197	221	205	166	259	170	185	170	197	134	109	146
22	252	202	170	163	160	176	211	122	160	259	149	193	107	99	115
23	226	172	168	251	250	224	244	224	167	184	122	120	136	126	131
24	261	147	195	211	262	200	210	165	237	193	219	178	135	106	101
25	282	168	208	259	295	270	229	216	202	225	200	175	126	95	99
26	243	165	250	227	255	259	253	239	209	220	183	176	156	162	208

Total sum of squares of all individual yields

$$\Sigma p^2 = 19714274$$

Mean of ultimate plots $\bar{p} = \frac{84128}{390} = 215{\cdot}713$.

The following table shows the coefficients of correlation obtained with the three different crops under trial in three different seasons :—

TABLE VI

Correlations for soil heterogeneity based upon the yields obtained from 390 ultimate plots, each 4 ft. square

Crop	Year	Coefficient of correlation ($r_{p_1 p_2}$)		Differences between the two coefficients of correlation and standard error
		1 × 5 combination	2 × 5 combination	
Barley Type 21	1929-30	0·4798 ± 0·0390	0·4769 ± 0·0391	0·0029 ± 0·0552
Wheat Pusa 52	1930-31	0·2361 ± 0·0468	0·2504 ± 0·0506	0·0143 ± 0·0689
Lentil Type 11	1931-32	0·5830 ± 0·0333	0·5921 ± 0·0329	0·0091 ± 0·0468

DISCUSSION OF RESULTS

Harris [1920] has pointed out that an exact measure of the influence of soil-heterogeneity is the first and most fundamental step in the closer analysis of the factors determining the variability of plot yields. If the application of such a criterion to results of uniformity trials shows no evidence of heterogeneity, plot tests may be carried out along conventional lines with confidence, and with reasonable precautions reliable results will be obtained. If, on the other hand, the application of such a criterion shows a high degree of irregularity in fields selected for their uniformity, it is evident that very special precautions must be taken to obtain trustworthy results. For a criterion of field heterogeneity Harris [1915] has suggested the coefficient of correlation of yields of contiguous plots. He believes that if a field sown with a single crop variety be divided into n small plots and if p denotes the yield of an individual plot, then the variability of p may be due purely and simply to chance, since the individuals of any variety are variable and the size of plots is small, or it may be due in part to the diversity of conditions of the soil. If the irregularities of the field are so large as to influence the yield of areas larger than single plots, they will tend to bring about a similarity of adjoining plots, some groups of plots tending to yield higher than the average, others lower. As heterogeneity becomes greater the correlation will also increase. The value of the coefficient obtained will depend somewhat upon the nature of the characters measured, the species grown, the size of the ultimate and combination plots, and to some degree upon the form of the combination plots.

In our experiments it should be noted that n was equal to 390 ultimate plots, each of these plots being four feet square. The combination plots in 1×5-fold grouping were therefore 4 ft. $\times$ 20 ft. in area and 78 in number ; while those in the 2×5-fold grouping were 8 ft. $\times$ 20 ft. in area and 39 in number.

The results shown in Table VI are of interest as they again bring out the fact that an experimental field which may be sensibly uniform for one crop or for one season may not be so for another crop or in a different season.

Whereas the coefficient of correlation for Pusa 52 wheat in 1930-31 is not very high for both the 1×5 and the 2×5-fold groupings, the coefficients are quite high in the case of barley in 1929-30 and lentils in 1931-32. Moreover, the coefficients of correlation in the two different kinds of grouping, in all the three crops under consideration, do not seem to be significantly different from one another, as is seen in the last column, where the differences of the two coefficients are in every case less than their respective standard errors. The coefficients of correlation in all cases appear to be significant when compared with their respective standard errors. The heterogeneous nature of the plot is therefore evident and the data will now be examined to see in what manner the soil-fertility varies in this field.

The means, standard deviations and the coefficients of variation obtained in these experiments and the standard errors of these constants are set forth in the table given below. These constants have been obtained by working with degrees of freedom equal to n—1 to keep the calculations in conformity with that followed in determining the constants for the heterogeneity coefficient. In this respect, as in others, Harris' method has been followed.

TABLE VII

Means, standard deviations, and coefficients of variation for the yields of barley, wheat and lentils

Crop	Year	Mean yields in grms.	Standard deviation	Coefficient of variation
Barley Type 21	1930	$634\cdot244\pm8\cdot166$	$161\cdot286\pm5\cdot775$	$25\cdot429\pm0\cdot968$
Wheat Pusa 52	1931	$251\cdot838\pm3\cdot730$	$73\cdot691\pm2\cdot637$	$29\cdot261\pm1\cdot136$
Lentil Type 11	1932	$215\cdot713\pm3\cdot217$	$63\cdot538\pm2\cdot274$	$29\cdot455\pm1\cdot143$

The means in this table show that the yields vary with the crop, the standard deviations naturally are influenced by the actual magnitudes of the yields and are therefore difficult of interpretation, whereas the coefficients of variation are more or less uniform. This suggests that the coefficient of variability is a more

reliable index than the mean and the standard deviation for judging the variability of a sample. In comparing "variations" under different sets of conditions or factors (as in dealing with different crops), therefore, the coefficient of variation is the most satisfactory index.

(2) Analysis of variance

The Latin square

All modern methods of field-experimentation have for their object the elimination of the factor of soil-heterogeneity. Of the more modern methods, Fisher's analysis of variance can be employed as an efficient tool for determining the 'drift' in the fertility of a given area. The plot yields obtained in the experiment under report have therefore also been analysed by applying this method. For the sake of convenience yields from the 26th row have been discarded and the whole area has been grouped into a 5×5 Latin square. Each individual plot of this Latin square therefore consists of fifteen ultimate plots, three running west to east by five lying north to south. The yields of these plots are shown in Table VIII.

TABLE VIII

Yields in decagrammes in grouped plots of barley, wheat and lentils

BARLEY TYPE 21				
1081·5	888·5	813·0	840·5	725·0
1218·5	1035·0	973·5	817·0	731·0
1216·0	1076·5	1055·5	825·0	693·5
1213·5	1091·5	1026·0	921·5	736·5
1147·0	1068·0	1067·5	764·5	606·5

WHEAT PUSA 52				
399·5	334·2	327·7	346·0	279·0
487·1	412·6	369·1	334·3	297·4
493·5	433·2	392·4	374·3	378·5
476·4	408·6	371·1	380·7	280·8
440·0	364·0	453·5	326·3	336·9

LENTIL TYPE 11				
462·6	339·5	302·5	310·2	320·3
513·7	414·7	353·8	305·9	249·8
440·5	373·4	308·8	257·2	223·0
387·1	371·3	263·5	262·4	226·2
319·9	334·4	298·2	276·9	176·5

The full arithmetical details of calculating the analysis of variance of these trials have not been given as such details are available in Fisher and Wishart [1930], Mahalanobis [1932] and Bose [1932] as well as in many other recent publications on the subject. The final analyses of variance are brought out in Table IX.

TABLE IX

Analyses of variance

Due to	Degrees of freedom	Sum of squares			Mean squares			$\frac{1}{2}\log_e$ Mean square		
		Barley	Wheat	Lentil	Barley	Wheat	Lentil	Barley	Wheat	Lentil
Columns . .	4	677558·00	57270·86	106223·44	169389·50	14317·71	26555·86	6·02001	4·78456	5·09341
Rows . . .	4	47814·30	15199·33	23801·82	11953·57	3799·83	5950·46	4·69442	4·12126	4·34558
Error . . .	16	61926·20	15586·88	19967·75	3870·39	974·18	1247·98	3·90027	3·44079	3·09614
Total . . .	24	787298·50	88057·07	149993·01	...	...	...	...	...	...
Standard error .	...	...	...	...	62·212	31·21	35·327	...	...	...

From the analyses of variance given above it will be noted that the differences of mean squares between columns and error are much greater than the differences of mean squares between rows and error. Applying Fisher's z-test for the one and five per cent levels of significance, we get the results shown in the following table :—

TABLE X

Differences between the $\frac{1}{2}\log_e$ (Mean square values) and their statistical significance

Crop	Values of z between		
	Row and error $n_1=4$ and $n_2=16$	Column and error $n_1=4$ and $n_2=16$	Column and row $n_1=4$ and $n_2=4$
Barley . .	0·79415 Significant at one per cent.	2·11974 Significant at one per cent.	1·32559 Not significant at one per cent but significant at 5 per cent.
Wheat . .	0·68047 Not significant at one per cent but significant at 5 per cent.	1·34377 Significant at one per cent.	0·66330 Not significant.
Lentil . .	0·78103 Not significant at one per cent but significant at 5 per cent.	1·52886 Significant at one per cent.	0·74783 Not significant.

From Fisher's table $z=0·7814$ for $P=0·01$ and $0·5505$ for $P=0·05$ when $n_1=4$ and $n_2=16$ and $z=1·3856$ for $P=0·01$ and $0·9272$ for $P=0·05$ when $n_1=4$ and $n_2=4$.

When the rows or columns are to be compared with error in the above table a difference greater than $0·7814$ for $n_1=4$ and $n_2=16$ is statistically significant. On the other hand if the rows and columns are to be compared with each other a difference greater than $1·3856$ for $n_1=4$ and $n_2=4$ is significant at the one per cent level. The results show a comparatively greater difference in fertility between the columns than between the rows. In this field, therefore, there is a fertility gradient from west to east running at right angles to the direction of the columns.

It may be pointed out that the analysis of variance method has been utilized in this case mainly to show the differences in soil-fertility and to show that a greater precision can be obtained in the standard error of the experiment by eliminating the variances due to soil-fertility.

Increased precision in the standard error may further be obtained in experiments such as these, where the same field or parts of the same field are employed

for two or more seasons in succession by utilizing Fisher's method of covariance for correcting the standard error of the experiment in the second or third year on the basis of that obtained in the first year.

The details of calculation for the reduction of the experimental error in the case of the present barley and wheat trials by the application of the covariance method have been provided in example 24 of Shaw's Handbook of Statistics for use in Plant Breeding and Agricultural Problems (*in Press*) and need not be repeated here. It is evident, however, that by taking preliminary yields into consideration increased precision to some extent may be obtained in the experimental error of the successive crop. Thus the mean square for error for Pusa 52 wheat is 974·18 as shown in Table IX and this could be reduced to 860·88 if the preliminary yields of barley in the same sub-plots are taken into consideration and the analysis of covariance is applied to this test. The precision of the comparison has thus been increased by $\frac{974 \cdot 18}{860 \cdot 88}$ or 1·13 times. Similarly, in the lentil trial, an improvement of 1·04 times can be effected in the standard error by applying the method of covariance. But as this hardly affects our results, we have not utilized these improved standard errors in our present study.

If we consider the yields of lentils obtained in the third year of the present experiment, we find that the yields range from 3952 grms. to 7832 grms. in the direction of the columns showing a coefficient of variability of approximately 21 per cent. The yields in the direction of the rows, on the other hand, range from 2638 grms. to 3880 grms. and have a coefficient of variation of only 11 per cent. Fig. 3 depicts these yields plotted out and their relation to the respective means of the total yields of columns and those of the rows. This shows again that there is a greater variation in the yields between columns than those between rows.

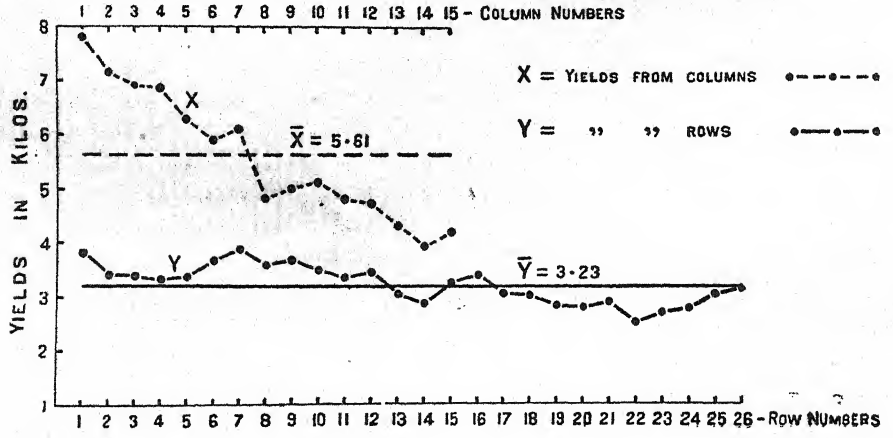


Fig. 3. Variation in yields of lentils from columns and rows.

Conclusions

A soil-heterogeneity experiment was conducted in a particular field for three consecutive years with barley, wheat and lentils respectively. After discarding the necessary borders the field was divided at harvest into 26 × 15 or 390 ultimate plots, each 4 feet square. The produce of each ultimate plot was separately harvested, hand-threshed and sun-dried. Weights were taken in grammes.

The coefficient of correlation between contiguous plots was employed as an index of soil-heterogeneity according to the method advocated by Harris. 1 × 5 and 2 × 5 combination plots were made up for this purpose by summing up the yields of either 5 or 10 contiguous plots; so that the 390 ultimate plots could be formed into 78 combination plots with the 1 × 5 grouping and 39 combination plots with the 2 × 5 grouping.

The coefficients of correlation for the two kinds of combinations are not significantly different from one another. For the different crops tried in three different years this constant was as follows :—

Crop	Year	Coefficients of correlation	
		1 × 5 combination	2 × 5 combination
Barley . . .	1929-30 . .	0·4798 ± 0·0390	0·4769 ± 0·0391
Wheat . . .	1930-31 . .	0·2361 ± 0·0468	0·2504 ± 0·0506
Lentils . . .	1931-32 . .	0·5830 ± 0·0333	0·5921 ± 0·0329

This shows clearly that an experimental field which is sensibly uniform for one crop in one season may not be so for another crop or in a different season, a result which is in conformity with those obtained by Harris in 1920. The presence of high correlation between contiguous plots signifies that, notwithstanding its uniform cultural and other treatments, the field has definite grades of fertility, which are so large as to influence the yields of areas larger than single combination plots in the field ; thus bringing about a similarity of adjoining plots, some groups tending to yield higher than the average, others lower.

Combinations of 2 × 3 ultimate plots were made and the field was considered as consisting of 65 such combination plots. Assuming the average yield of each plot to be at the centre, the points at which the yields were 10, 20, 30, 40, etc., per cent above or below the mean yield were found by interpolation between adjacent plots. These points were joined and the contour maps shown on page 580 were constructed. It is evident that the yield varied greatly between different

plots in the same field. That this heterogeneity was systematic to a considerable extent is also apparent as the fertility contour lines run more or less parallel to the columns (north to south) to a very pronounced degree [Bose, 1933]. The contour lines show a good deal of similarity in all the three maps shown above. The differences are due, of course, to the variability in the yielding power of the different crops under consideration and their relation to the mean yields of each.

It has also been found that, although the mean yields in this field have varied widely with the crop, the coefficients of variation have remained more or less uniform in the three years under report. Barley, for instance, gave a mean yield of about 634 grms. per ultimate plot, wheat has given only about 251 grms., and lentils about 216 grms. The coefficients of variability in these crops were 25·4, 29·3, and 29·4 per cent, respectively.

Fisher's analysis of variance method is one of the best tools for determining the drift in the fertility of a given area. The plot yields obtained in these experiments were therefore analyzed by applying this method also. Discarding the yields of the last or the 26th row, it is possible to compose a 5×5 Latin square with the yields of the remaining ultimate plots. On analysis it is found that there is a statistically significant difference between the variance for 'columns' and that for 'error' and also between the variance for 'rows' and 'error'. From this it may be inferred that fertility gradients run both along rows and along columns.

It may be pointed out that Harris' method of determining soil-heterogeneity by the estimation of the coefficient of correlation between contiguous plots provides a measure for heterogeneity present in the whole field. On the other hand, Fisher's analysis of variance method which has come into existence long after Harris' method, not only provides a measure of soil-heterogeneity but also clearly sets forth the direction of the fertility slope. The latter method should, therefore, furnish a more comprehensive estimate of variations in fertility in different parts of a field.

In agricultural experimental stations where the testing of improved varieties of crops or their response to different manurial treatments forms an important item of work, a knowledge of the uniformity of different fields proves very advantageous in the guidance that it usually gives in designing future lay-outs on such fields. All that is required in this connection is that each year one or two fields which are under any bulk crop in the station may be harvested in the form of small ultimate units of convenient size and shape. The yields of these ultimate plots will furnish sufficient evidence to determine the nature of the heterogeneity of the field and also of the direction of the fertility gradient present therein. From this study the correct size and shape of plots that would furnish reliable results can also be determined. The threshing of these separate small units would undoubtedly entail some extra expenditure, but this would be amply compensated for by obtaining a reliable index of the nature of the field for further trials.

Statistical methods, it must be remembered, furnish trustworthy results only if they are employed in the right way to properly controlled field experiments [Bose, 1933]. If an experiment is wrongly placed no method can be expected to give significant results.

II. THE SIZE AND SHAPE OF EXPERIMENTAL PLOTS

The existence of soil-heterogeneity in an experimental plot at Pusa has been shown in the first part of this paper and it has been pointed out that one of the chief advantages of estimating soil-heterogeneity in experimental fields is that this furnishes material on which to base the proper lay-out and to determine the correct size and shape of plots necessary to reduce errors due to soil-fertility, etc., to a minimum. Should the yields of ultimate units in a field be known, it is possible to combine these in various ways so as to get lay-outs with different plot sizes and plot shapes and the one which shows the least standard error may be gauged to be the best for the conditions of the experiment. Fisher's analysis of variance may be used for determining the standard errors of the various combinations. The results obtained in the wheat trial reported in the preceding pages may be employed to furnish some idea of the actual arithmetical details necessary.

Studies to determine the most efficient size and shape of plot have been conducted with a great variety of crops and under different sets of conditions. Even then there is a great scope for this kind of work under varied conditions of soils and climate that obtain in India. In their classical experiments, Mercer and Hall [1911] calculated the probable errors for combinations of different sizes and number of replications of small unit plots, each 1/500 of an acre in area, and found that the error diminished with the size of the plot, but that the reduction was small when the plots grew above 1/40th of an acre. Parnell [1919] found that an area of 50 × 4 feet repeated eight times would reduce the probable error of mean difference in paddy to about two per cent. Iliffe and Srinivasan [1926] observed that a 40 × 5 feet plot replicated six times reduced the probable error of the difference in paddy to such an extent that a 10 per cent difference in the experiments would be significant.

Immer [1932] studied the size and shape of plots in relation to field-experiments with sugar-beets and found that the standard errors, expressed as percentages of the mean, decreased in general with increased size of plot, and that the experimental efficiency of the land decreased with increased size of plot when the entire plot was harvested. When the border rows of the plots were removed 4-row plots were most efficient.

A complete bibliography bearing on this subject has been published by a committee of the American Society of Agronomy for the standardization of field-experiments in the *Journal of the American Society of Agronomy*, **18**, 1926, pages 1143—1144 ; **22**, 1930, pages 1056—1061.

In plant-breeding work, where numerous strains are compared, the size and shape of plot is necessarily limited by available space and sometimes by the amount of seed. In comparative yield trials, however, where only a few varieties are being compared, it is always possible to lay out plots of such size and shape as will reduce the errors due to variation in soil-fertility to a minimum. This is easily accomplished if an idea of soil-heterogeneity in the experimental field under observation is available.

The data obtained from the uniformity trial with Pusa 52 wheat has been employed for the purpose of determining the best size and shape of plot which could be oriented in this particular field for future experiments and which would reduce the experimental error to its minimum. In Table IV have been presented the yields of 26 × 15 ultimate plots of Pusa 52 wheat, but for the sake of convenience the ultimate plots in the last two rows have been omitted for the present study and the yields of 24 × 15 or 360 ultimate units only have been taken into consideration. By combining plot yields of various numbers of adjacent ultimate units combination blocks of different sizes and shapes can be formed. Fisher's analysis of variance may be used and five hypothetical treatments may be assumed in order to make due allowance for soil-heterogeneity. Thus, in each combination or lay-out the total variance is split up into variance due to (1) 'between blocks' and (2) 'within blocks'. The variance due to the last item gives as its square root the standard deviation of the combination plots from which the coefficient of variation can be computed.

Taking the 3 × 3 combination as an illustration, we find that the experimental field could be divided into 40 sub-plots, each 12 ft. × 12 ft. in size, and in which the yields of three ultimate units in the direction of the rows and of three units in the direction of the columns have been combined to represent the yield of each sub-plot. On the assumption of five hypothetical treatments we can thus divide the field into 40/5 or 8 blocks. The plot yields of this lay-out are shown on the margin. The total sum of squares for calculating the analysis of variance is obtained by squaring the yield of each sub-plot, summing and subtracting from this the correction factor which is obtained by dividing the square of the general total by the total number of combination plots—in this case 40.

Block						Block total
I	2178	1995	1835	2060	1570	9638
II	2738	2078	2022	2030	1742	10610
III	2938	2568	2319	1881	1792	11495
IV	3127	2402	2412	2137	2190	12268
V	2818	2760	2304	2438	2255	12575
VI	2774	2332	2169	2177	1683	11135
VII	3029	2549	2329	2445	1880	12232
VIII	2571	2230	2708	1938	2009	11456
				General total		91409

$$\text{Correction factor} = \frac{(91409)^2}{40} = 208890132{\cdot}025$$

Plot and block yields in 3 × 3 combination.

The sum of squares 'between blocks' is computed by squaring the block yields, summing these up and dividing this sum by the number of blocks, and finally, subtracting the same correction factor as was used in obtaining the true total sum of squares. The sum of squares due to variations 'within blocks' is therefore the difference between the total sum of squares and that due to variations 'between blocks'. The analysis of variance for the 3 × 3 combination is tabulated below :—

TABLE XI

Analysis of variance for 3 × 3 combination plots

Source of variation	Degrees of freedom	Sum of squares	Mean square	Significance or ratio of $\frac{\text{Variance}}{\text{Variance 2}}$
Between blocks . . .	7	1326388·575	189484·082	1·403
Within blocks	32	4319824·400	134994·510	Expected 3·47
Total .	39	5646212·975		

Standard deviation $= \sqrt{134994 \cdot 510} = 367 \cdot 41$

Co-efficient of variation $= \frac{\sigma \times 100}{\text{Mean}} = 16 \cdot 078$ per cent.

In this particular case the observed ratio of variance 1 to variance 2 being smaller than the expected ratio, no particular gain has been made by eliminating the variance due to soil heterogeneity, but this is not so in all the combinations used in this study.

Fig. 4 shows a diagrammatic representation of some of the combinations that can be formed on this experimental field on the assumption of five hypothetical treatments and the figures at the bottom of each lay-out show the number of ultimate units that have been included to form each combination plot in that series.

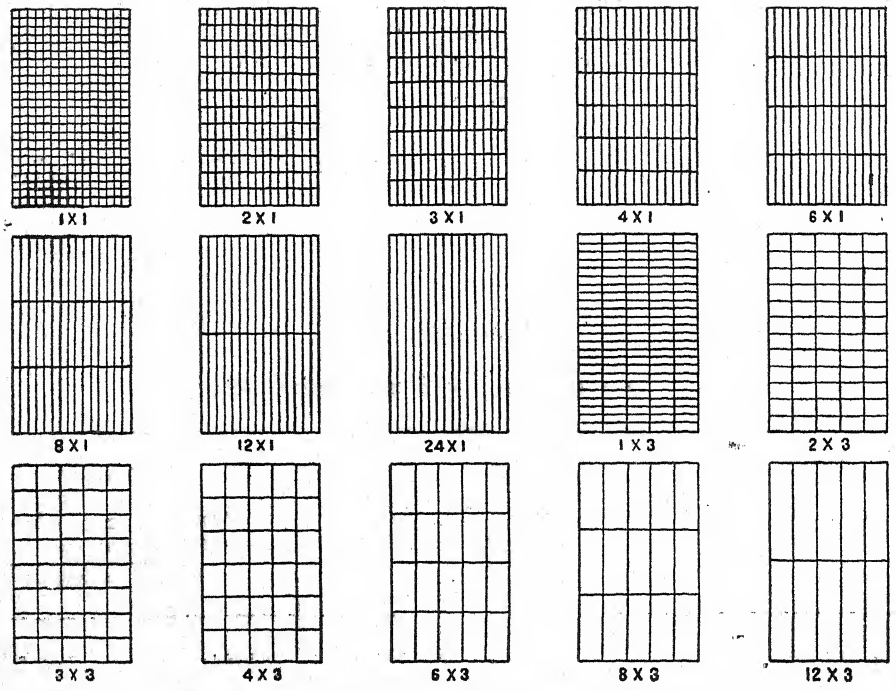


Fig. 4. Combination plots of various sizes and shapes.

Table XII depicts the analyses of variance in different combinations of Pusa 52 wheat, together with the coefficients of variations ' within plots ' for each.

TABLE XII

Analyses of variance in different combinations—wheat uniformity trial 1930-31

Combination series	Size of sub-plots	No. of sub-plots in each combination	Variation	Degrees of freedom	Sum of squares	Mean square	Ratio of variance 1 to variance 2		Variation 'within blocks'.	
							Observed	Expected at one per cent level	Standard deviation	Co-efficient of variation
										Per cent
1 × 1	4 ft. × 4 ft.	360	Between blocks	71	647869·53	9124·923	2·284	1·14	63·21	24·894
			Within blocks	288	1150630·80	3995·246				
2 × 1	8 ft. × 4 ft.	180	Between blocks	35	1114642·46	31846·927	2·835	1·27	105·99	20·871
			Within blocks	144	1617633·20	11233·564				
3 × 1	12 ft. × 4 ft.	120	Between blocks	23	1636175·76	71138·076	3·311	1·98	146·56	19·240
			Within blocks	96	2062162·40	21480·858				
4 × 1	16 ft. × 4 ft.	90	Between blocks	17	1995965·12	117409·713	3·403	2·45	185·74	18·288
			Within blocks	72	2483861·20	34498·072				
6 × 1	24 ft. × 4 ft.	60	Between blocks	11	2814670·58	255879·143	3·928	2·56	256·05	16·807
			Within blocks	48	3146996·40	65562·425				
8 × 1	32 ft. × 4 ft.	45	Between blocks	8	3700454·44	462556·805	4·269	3·07	329·13	16·204
			Within blocks	36	3899885·20	108330·140				
12 × 1	48 ft. × 4 ft.	30	Between blocks	5	4528666·43	905733·286	4·060	3·90	472·30	15·501
			Within blocks	24	5354266·20	223068·050				

TABLE XII—*concld.*

Combination series	Size of sub-plots	No. of sub-plots in each combination	Variation	Degrees of freedom	Sum of squares	Mean square	Ratio of variance 1 to variance 2		Variation 'within blocks'	
							Observed	Expected at one per cent level	Standard deviation	Co-efficient of variation
										Per cent
24 × 1	96 ft. × 4 ft.	15	Between blocks	2	8146081·87	4073040·93	6·958	6·93	765·10	12·555
			Within blocks	12	7025064·40	585422·03				
1 × 3	4 ft. × 12 ft.	120	Between blocks	23	592821·76	25774·86	1·285	1·98	141·59	18·590
			Within blocks	96	1924150·40	20043·34				
2 × 3	8 ft. × 12 ft.	60	Between blocks	11	862825·78	78438·70	1·167	2·56	291·89	19·159
			Within blocks	48	3226927·20	67227·65				
3 × 3	12 ft. × 12 ft.	40	Between blocks	7	1326388·57	189484·08	1·403	3·47	367·41	16·078
			Within blocks	32	4319824·40	134994·51				
4 × 3	16 ft. × 12 ft.	30	Between blocks	5	1239026·17	247805·23	1·079	3·90	465·83	15·722
			Within blocks	24	5508004·80	229500·20				
6 × 3	24 ft. × 12 ft.	20	Between blocks	3	1809159·35	603053·12	1·287	5·29	684·52	14·975
			Within blocks	16	7497123·60	468570·23				
8 × 3	32 ft. × 12 ft.	15	Between blocks	2	2269997·73	1134998·87	1·450	6·93	884·66	14·508
			Within blocks	12	9391625·20	782635·43				
12 × 3	48 ft. × 12 ft.	10	Between blocks	1	1147176·90	1147176·90	1·488	11·26	1306·50	15·387
			Within blocks	8	13655638·00	1706954·75				

It will be noted from the above table that the range of the coefficients of variability extends from 12·555 per cent for the 24 × 1 combination to 24·894 per cent for the 1 × 1 combination. The best combinations that can be used for future experiments in this field appear to be 24 × 1, 12 × 3, 8 × 3 and 6 × 3, as these give the least values for their respective coefficients of variation. It must be remembered, however, that for cereals in general, a plot size approximating 1/40th of an acre is said to be ideal but in this country a good deal of work appears to be necessary to fix up the correct size of plots for different crops under the different sets of soil conditions that are met with.

Shape of plots

An inspection of Fig. 4 will show that by taking various combinations of ultimate units, combination sub-plots of various sizes and shapes can be formed. Of these lay-outs 8 × 3 and 24 × 1 have the same plot size but different shapes and these two lay-outs also exhibit some difference in their coefficients of variation. Similarly, there are other combinations which possess the same size of plots but which differ in their shapes and in their coefficients of variability. These are enumerated below :—

TABLE XIII

Showing the difference in the coefficients of variations in plots of equal size but of different shapes

Lay-out	No. of ultimate units to form each combination plot	Size of each combination plot in sq. feet	Co-efficient of variation	Difference in co-efficient of variation
8 × 3	15	384	14·508	1·953
24 × 1	15	384	12·555	
4 × 3	30	192	15·722	0·221
12 ×	30	192	15·501	
2 × 3	60	96	19·159	2·352
6 × 1	60	96	16·807	
1 × 3	120	48	18·590	0·650
3 × 1	120	48	19·240	

These figures provide ample evidence to bring out the importance of the shape of the experimental plots in a given area. Plots of the same size but of different shapes have shown differences in their variability. This is chiefly due to the presence of various grades of soil fertility in the field and the inclusion of patches of unequal fertility in combination plots of varying shapes.

Lay-outs 8 × 3 and 24 × 1, for instance, are such that both divide the experimental area into fifteen equal divisions. The size of plots as well as the number of ultimate units taken to form each plot are also equal in both cases. The shape of the plots, however, differ in that in the 24 × 1 combination the field has been divided longitudinally in the direction of the columns, that is, from the north to the south, and the length of each plot is many times its breadth. In the 8 × 3 combination, on the other hand, the field has been divided into fifteen compact plots in which the ratio of length to breadth is not very great. The difference in the shape of plots in the two lay-outs has brought in a difference of 1·953 in their coefficients of variation, thus showing the superiority of the 24 × 1 arrangement over the other. It has been pointed out in the first part of this paper that the fertility gradient in this experimental field lies from west to east and that combination plots taken at right angles to this direction, *i.e.*, lying from north to south would give a smaller variability than those lying in the direction of the fertility slope. It is probably for this reason that the 24 × 1 combination, in which the plots lie in the direction of north to south, has provided the least variability.

Should the fertility slope in any experimental field be known, it is possible, then, to predict what shape as well as what size of plots would furnish the most reliable results.

Summary

Results are reported of soil-heterogeneity experiments conducted for three consecutive years in the same field with Pusa barley, wheat and lentils.

The coefficient of correlation between contiguous plots was employed as an index of soil heterogeneity according to Harris' method, and 1 × 5 and 2 × 5 combination plots were made up for this purpose. Although the coefficients of correlation for these two kinds of combinations were not significantly different from one another, the presence of significant coefficients for each combination denotes definitely that the field under consideration was not absolutely uniform.

Fisher's analysis of variance was also employed to determine the drift in the fertility of the field with the same data. It was found that there was a great deal of variation in the yields for columns and much less in the rows, suggesting that there was a fertility gradient in this particular field which ran from west to east. This was further seen when contour maps of soil fertility were drawn from results of plot yields.

Harris' method of determining soil heterogeneity provides a measure of heterogeneity present in the whole field, but Fisher's analysis of variance not only provides a measure of soil heterogeneity but also clearly sets forth the direction of the fertility gradient and should, therefore, be a more comprehensive method for such work.

A knowledge of the amount and direction of variability in the fertility of any experimental field helps in the proper laying out of yield or manurial trials in the right direction and gives a measure of the shape and size of plot which ought to be employed. The combination which yields the least coefficient of variation within blocks in a preliminary trial is to be taken as the best one for laying out future trials in a field. The results of the uniformity trial conducted with Pusa 52 wheat have been utilized to show what size and shape in this field will produce the least variation 'within plots' on the assumption of five hypothetical treatments. The analysis of variance method is used for the interpretation of these results.

The writer takes great pleasure in expressing his indebtedness to Dr. F. J. F. Shaw, Imperial Economic Botanist, Pusa, and now Director of the Imperial Institute of Agricultural Research, under whose guidance this study was made, and to Messrs. M. B. V. Narasingha Rao and R. G. Joglekar, Post-graduate students in Botany, for help given during the course of the study. Thanks are also due to Rao Bahadur M. Vaidyanathan, Statistician, Imperial Council of Agricultural Research, for helpful criticisms and suggestions.

REFERENCES

Bose, R. D. (1928). *Mem. Dept. Agric. Ind., Bot. Ser.* **16**, 159-189.

———— (1931). *Ind. J. Agric. Sci.* **1**, 58-89.

———— (1933). *Agric. and Live-stock in India* **3**, 330-340.

Eden, T. (1931). *J. Agric. Sci.* **21**, 547-573.

Fisher, R. A. (1932). 'Statistical Methods for Research Workers'. Oliver and Boyd, London.

Fisher, R. A. and Wishart, J. (1930). *Imp. Bur. of Soil Sci. Tech. Communication* No. **10**.

Garber, R. J.; McIlvaine, T. C.; and Hoover, M. M. (1926). *J. Agric. Res.* **33** 255-268.

Hall, A. D. (1909). *J. Bd. Agric., London* **16**, 365-370.

Harris, J. A. (1915). *Amer. Nat.* **49**, 430-454.

———— (1920). *J. Agric. Res.* **19**, 279-314.

Howard, A. and Howard, G. L. C. (1927). *Agric. Res. Inst. Pusa Bull.* No. **171** p. 22.

Iliffe, R. O. and Srinivasan, C. R. (1927). *Annual Report of the Paddy Breeding Station, Maruteru, Madras*, 1926-27, 1-31.

Immer, F. R. (1932). *J. Agric. Res.* **44**, 649-668.

Lehmann, A. (1901-1908). *Second to Ninth Annual Reports of the Agricultural Chemist, Dept. of Agric., Mysore State (India)*, 1900-01 to 1907-08.

Lord, L. (1931). *J. Agric. Sci.* **21**, 178-188.
Mahalanobis, P. C. (1932). *Ind. J. Agric. Sci.* **2**, 686-689.
Mercer, W. B. and Hall, A. D. (1911). *J. Agric. Sci.* **4**, 107-132.
Montgomery, E. G. (1911). *Nebraska Agric. Expt. Stn. 25th Ann. Rept.*, 164-180.
Parnell, F. R. (1919). *Agric. J. Ind.* **14**, 747-757.
Sanders, H. G. (1930). *J. Agric. Sci.* **20**, 63-73.
Shaw, F. J. F. (1935). 'Handbook of Statistics for Use in Plant-Breeding and Agricultural Problems'. (*In Press*).
Shaw, F. J. F. and Bose, R. D. (1929). *Agric. J. Ind.* **24**, 373-396.
Wood, T. B. and Stratton, F. J. M. (1908). *J. Agric. Sci.* **3**, 417-440.

INFLUENCE OF TEMPERATURE AND MATURITY ON THE INCIDENCE OF SANN-HEMP AND PIGEON-PEA WILT AT PUSA

BY

B. B. MUNDKUR

Assistant Mycologist, Imperial Institute of Agricultural Research, Pusa

(Received for publication on 25th February 1935)

(With two text-figures)

CONTENTS

Introduction

In a recent article Mitra [1934] has shown that the wilt disease of sann-hemp, *Crotalaria juncea* Linn., is caused at Pusa by *Fusarium vasinfectum* Atkinson, and that the isolates of this fungus from wilted sann-hemp plants produce wilt in pigeon-pea, *Cajanus indicus* Sprengel, and *vice versa*. At Pusa at any rate the same *Fusarium* sp. has been found to cause wilt in these two crops. An attempt is made in this paper to show the close association existing between soil temperature and the maturity of plant and the incidence of *Fusarium* wilt in these two crops.

Numerous investigations dealing with the influence of soil temperature on plant diseases have been reported, and the literature has been exhaustively summarised by Jones, Johnson and Dickson [1926]. A review is therefore unnecessary

here except to emphasize that temperature alone is not the limiting factor of environment in the occurrence of plant diseases, but such factors as soil moisture, soil reaction and soil texture are intimately concerned and operate as a whole in limiting or favouring the causation of plant diseases. Furthermore, Jones *et al* have concluded that most of the vascular *Fusarium* wilts are favoured by soil temperatures in the neighbourhood of 24-28°C. and that the disease development-temperature curves in most cases parallel the fungus growth-temperature curves.

The work at Wisconsin and other centres was done in carefully controlled soil temperature tanks, but the results discussed in the present paper are based entirely on field data. In addition to showing that the wilt diseases of sann-hemp and pigeon-pea are influenced by soil temperature, these results indicate that the same fungus causes wilt in these two crops under two entirely different sets of conditions. In sann-hemp the incidence of wilt is favoured by soil temperatures ranging approximately from 28 to 33°C., while in pigeon-pea the occurrence of wilt is favoured by slightly lower temperatures, between 17 and 29°C., recalling the case of *Giberella saubinettii* (Mont) Sacc., which, according to Dickson [1923], causes a severe blight in wheat at relatively high temperatures, 16-28°C., and in maize at comparatively low temperatures, 16-20°C.

Maturity of plants appears to exert a good deal of influence on the incidence of wilt in the two crops under discussion as shown by the correlation coefficients that have been calculated.

Materials and methods

Pigeon-pea and sann-hemp plants were sown in a one-tenth acre plot in alternate rows on the 14th of June 1933. The pigeon-pea used was Pusa Type 5 which has been reported by McRae and Shaw [1933] to be highly susceptible to *Fusarium* wilt. The seed of sann-hemp was secured from the Imperial Agriculturist who had collected it during the previous year from a field of sann-hemp where wilt was rather severe. The plot where these pigeon-pea and sann-hemp types were sown has, since 1923, been developed into a highly wilt-infested plot by the addition, every year, of cut pieces of wilted pigeon-pea plants and ploughing in the land so as to mix the material intimately with the soil. At the present time if a susceptible type of pigeon-pea is sown in it, hardly a plant survives at the end of the season. There were in all 490 plants of sann-hemp and 468 of pigeon-pea in the plot.

The soil temperatures were obtained by using an automatic earth temperature recorder that had been previously standardised by comparing it with a standard thermometer. Its bulb was buried at a depth of six inches in the field. The weekly mean temperatures were obtained by summating the temperatures recorded at every two-hour intervals during the week on the recorder sheets and dividing the total by 84, the total two-hour periods in the week. The well-known Pearsonian equations of simple, multiple and partial correlation were used in calculating the values of the respective coefficients.

On a fixed day in each week the plot was carefully examined and the dead plants were counted and removed. These were brought to the laboratory and each plant examined to see if the death was due to *Fusarium* wilt or *Rhizoctonia* wilt. No account was taken of the plants that had died as a result of *Rhizoctonia* attack in these calculations. The weekly percentages were calculated on the basis of the number of deaths in each week to the number living at the beginning of the particular week.

Analysis of first set of data

Simple correlation between wilt incidence and soil temperature

In Table I are recorded the weekly percentage deaths of sann-hemp and pigeon-pea plants and the weekly mean soil temperatures.

Table I

Percentage of weekly deaths of sann-hemp and pigeon-pea plants due to wilt

Age in weeks	Weekly mean soil temperature (°C.)	Sann-hemp		Pigeon-pea	
		Actual deaths	Percentage of deaths	Actual deaths	Percentage of deaths
8	34	2	..	..	..
9	33	7	1·5	..	..
10	29	20	4·4	2	..
11	29	20	4·6	10	2·1
12	30	87	21·2	5	1·1
13	31	101	31·1	10	2·2
14	31	87	16·5	8	1·8
15	29	36	19·3	22	5·1
16	29	59	39·1	28	6·8
17	29	19	20·7	36	9·4
18	29	16	22·2	27	7·8
19	28	10	17·5	30	9·4
20	24	13	27·7	44	15·2

Age in weeks	Weekly mean soil temperature (°C.)	Sann-hemp		Pigeon-pea	
		Actual deaths	Percentage of deaths	Actual deaths	Percentage of deaths
21	24	1	3·0	18	7·3
22	24	4	12·1	18	7·9
23	22	5	17·2	64	30·5
24	21	2	8·3	33	22·6
25	20	0	0·0	30	26·5
26	20	0	0·0	20	24·1
27	17	0	0·0	17	27·0
28	17	1	4·7	14	30·4
29	18	..	..	6	18·8
30	18	..	..	4	19·2

After the 8th of January 1934 deaths ceased in the plot and no further readings were recorded. An examination of the data will show that while sann-hemp plants died in the earlier part of the season, those of pigeon-pea succumbed only in the latter part.

In order to determine the nature and magnitude of the correlation existing between soil temperature and wilt incidence in sann-hemp plants, the simple coefficient of correlation was first calculated. The value of '*r*' was found to be +0·537. There are twenty observations (column 4) and two variables so that the degrees of freedom are eighteen. At five per cent level of significance, the significant value of '*r*' for that number of degrees of freedom, according to Fisher [1932], is 0·444. As the value actually obtained is higher than this, it is clear that as temperature increases from 17 to 33°C., deaths due to *Fusarium* wilt in sann-hemp also increase.

A similar coefficient of correlation was also obtained for wilt incidence and soil temperature in pigeon-pea plants. The value of '*r*' in this case was found to be —0·901. This is a high value showing a very close association between soil temperature and wilt incidence in pigeon-pea plants. Apart from this, it will be noted that there is a negative sign preceding the coefficient which is indicative of the fact that wilt incidence in pigeon-pea plants at a range of temperature of 17 to 33°C. decreases as the temperature rises. In other words, the effect of soil temperature in the case of sann-hemp is to increase wilt incidence as the temperature

increases and quite the reverse in the case of pigeon-pea plants where wilt decreases as the temperature increases (always of course within the range of 17 to 33°C.).

Simple correlation between wilt incidence and maturity of the host

Examination of data also made it apparent that there may be some association between the maturity of plants (*i.e.*, their age in weeks) and the incidence of wilt. Simple coefficient of correlation was therefore calculated and the values of '*r*' obtained in the two cases are given below :—

Simple coefficient of correlation between maturity of sann-hemp plants and incidence of wilt . . . . . —0·314

Simple coefficient of correlation between maturity of pigeon-pea plants and incidence of wilt . . . . . +0·941

It will be noted that the value obtained in the case of sann-hemp does not show association being much smaller than the significant value, *viz.*, 0·444. It is negative, showing that these plants lose their susceptibility to the disease as they mature. In pigeon-pea there is very close association between maturity and wilt incidence, and as the positive sign precedes the value, the plants seem to lose their resistance to the wilt disease as they age and become more and more vulnerable to attack.

Finally, in order to show graphically the relationships of the data recorded in the table, the regression equation of soil temperature on wilt incidence and that of maturity on wilt incidence were calculated. The equations are given below and the regression lines indicating the trends are shown in Figs. 1 and 2.

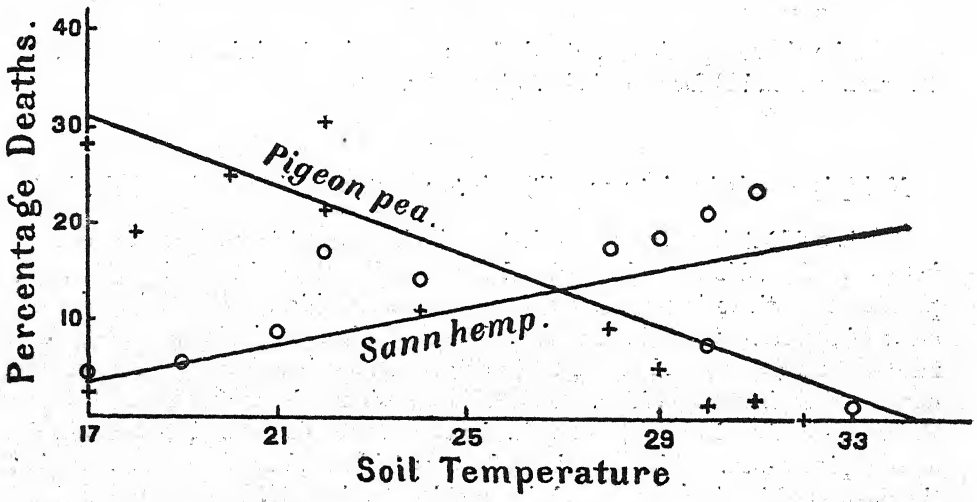


Fig. 1. Regression line showing relation between soil temperature and incidence of wilt.

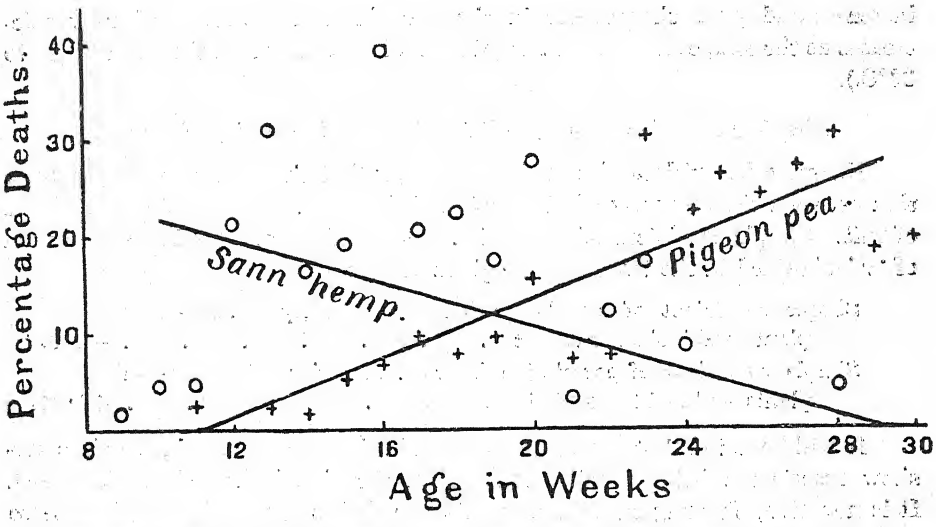


Fig. 2. Regression line showing relation between maturity and incidence of wilt

Regression of wilt incidence on soil T° in pigeon-pea	$X = 61{\cdot}7292 - 1{\cdot}795A$
Regression of wilt incidenceon soil T° in sann-hemp	$X = 0{\cdot}9375A - 12{\cdot}103$
Regression of wilt incidence on maturity in pigeon-pea plants	$X = 1{\cdot}477B - 16{\cdot}098$
Regression of wilt incidence on maturity in sann-hemp plants	$X = 33{\cdot}266 - 1{\cdot}1307B$

In the above equations X stands for per cent deaths, A for weekly mean soil temperature and B for age of plants in weeks.

Multiple correlation between wilt, soil temperature and maturity of the host

It is evident from the above that, while the attempt to analyse the relations between wilt incidence and associated variables has shown interesting facts, it is possible that there is a regular network of relationships. The multiple coefficient of correlation existing between these variables, wilt incidence ($= X$), soil temperature ($= Y$) and maturity ($= Z$), was therefore determined to see the nature of such relationships. This value, designated by the letter 'R' by Pearson, was found to be $+ 0{\cdot}951$ in the case of sann-hemp and $+ 0{\cdot}943$ in the case of pigeon-pea. There are here three variables and 23 observations, so that the degrees of freedom are 20 and the significant value of R at one per cent level of significance is $0{\cdot}608$ according to Table 16 of Wallace and Snedecor [1931]. These values indicate high association between these variables.

Partial correlation between the vriables

Finally it remained to determine whether the correlation between soil temperature and wilt continued to exist if the influence of maturity was eliminated, or between maturity and wilt if the influence of soil temperature was eliminated. Correlation coefficients between the two variables, independent of the third variable, were therefore calculated by determining the first order partial correlation coefficient. The values are given below :—

First order coefficient of correlation between soil temperature and wilt, independent of maturity in sann-hemp . .	= + 0·459
First order coefficient of correlation between soil temperature and wilt, independent of maturity in pigeon-pea . .	= — 0·485
First order coefficient of correlation between maturity and wilt, independent of temperature in sann-hemp . . .	= — 0·437
First order coefficient of correlation between maturity and wilt, independent of temperature in pigeon-pea . . .	= + 0·278

Here again the degrees of freedom are 20 and the significant value at one per cent level of significance is 0·608 and at five per cent level of significance 0·509, according to Wallace and Snedecor [1931]. None of the values is significant biometrically. It is apparent therefore that the influence of soil temperature and maturity on the incidence of wilt in sann-hemp and pigeon-pea at Pusa is a combined influence and not due to any one of them alone.

Analysis of a second set of data

Data from another set of experiments were also available to test the hypothesis advanced in the previous paragraphs. In a small plot which had not been either under sann-hemp or pigeon-pea for a number of years, the soil was heavily infested with pieces of wilted sann-hemp plants collected the previous year. These pieces were intimately mixed with the soil and ploughed in before the rains in 1933. Same types of pigeon-pea and sann-hemp were sown on the 14th of June 1933. There were 503 sann-hemp plants and 278 pigeon-pea plants. Dead plants of both the crops were counted and removed every week as before. Plants which showed under the microscope that they had succumbed to *Rhizoctonia* attack have not been included in these calculations. In Table II are recorded the soil temperatures and the percentages of wilt attack, calculated as before.

TABLE II

Percentage of weekly deaths of sann-hemp and pigeon-pea plants due to wilt

Age in weeks	Weekly mean soil temperature (°C.)	Sann-hemp		Pigeon-pea	
		Actual deaths	Per cent	Actual deaths	Per cent
8	34	24	4·8	7	2·4
9	33	31	6·5	4	1·4
10	29	49	10·9	3	1·0
11	29	74	18·5	5	1·8
12	30	133	40·9	7	2·6
13	31	70	36·5	15	5·7
14	31	25	20·5	12	4·9
15	29	18	18·6	6	2·6
16	29	13	16·5	19	8·3
17	29	24	36·4	4	1·9
18	29	10	23·8	4	1·9
19	28	1	3·1	6	3·0
20	24	4	12·9	9	4·6
21	24	4	14·0	0	0·0
22	24	4	17·3	3	1·6
23	22	2	10·0	4	2·2
24	21	2	11·1	7	3·9
25	20	0	0·0	2	1·2
26	20	0	0·0	2	1·2
27	17	0	0·0	2	1·2
28	17	0	0·0	3	1·8
29	18	0	0·0	5	3·1
30	18	0	0·0	6	3·8
31	17	0	0·0	16	10·5
32	13	0	0·0	11	8·1
33	14	0	0·0	5	4·0
34	15	0	0·0	27	22·5

It will be noted, from the data recorded in Table II, that 488 out of a total of 503 sann-hemp plants succumbed to the disease, while 194 pigeon-pea plants out of a total of 287 were attacked. The first flush of deaths in the case of the former crop took place in the earlier part of the season when soil temperatures were rather high and plants were comparatively young. The deaths ceased entirely after the 24th week. In the case of pigeon-pea the plants succumbed throughout the period, more deaths however taking place in the later than in the earlier part of the season and at a time when soil temperatures were comparatively low.

In order to see the numerical relationship of these associations, the simple coefficients of correlation were also calculated, and the values are given below.

Coefficient of correlation between soil temperature and wilt incidence in sann-hemp . . . . . = + 0·170

Coefficient of correlation between wilt incidence and soil temperature in pigeon-pea . . . . . = — 0·366

Coefficient of correlation between wilt incidence and maturity in sann-hemp . . . . . = — 0·104

Coefficient of correlation between wilt incidence and maturity in pigeon-pea . . . . . = + 0·347

It will be observed that none of the values is significant, which is manifestly due to the fact that as the plot, which was not naturally infected, had been but recently artificially infested with the wilt pathogen, its index of infectivity of the soil was not as high or as uniform as that of the former plot. But the complete absence of deaths after the 24th week in the sann-hemp crop and the deaths that continued to take place in pigeon-pea support in a general way the hypothesis advanced in the first part of this paper.

Discussion of results

Data have been presented in the preceding pages which prove that there is a close correlation between soil temperatures and the occurrence of wilt in the two leguminous crops, sann-hemp and pigeon-pea. It has been further shown that while in the former crop high temperatures favour the disease, low temperatures seem to favour it in pigeon-pea. This hypothesis has been further supported by the presentation of evidence derived from a second set of experiments.

Jones, Johnson and Dickson [1926] are inclined to the view that in the case of vascular *Fusarium* wilts soil temperatures in the neighbourhood of 24-28°C. favour the disease. While this may be true in the cases investigated by them, that does not seem to be so in sann-hemp.

In addition to soil temperature, the maturity of plants appears to exercise some influence on the incidence of wilt. The simple coefficients of correlation between wilt incidence and maturity of sann-hemp and pigeon-pea plants gave values of '*r*' which showed that while in the former crop this was partly true, it was a significant relationship in the latter case.

When multiple correlation coefficients were determined in the two cases, high values were again obtained, but the first order partial coefficients of correlation, using the data in the first table, showed that the influence of soil temperature and maturity on the incidence of wilt is not due to either of these acting independently but that it is a combined influence.

SUMMARY

1. Weekly death records in sann-hemp and pigeor-pea due to wilt caused by *Fusarium vasinfectum* Atkinson indicated that high soil temperatures, between 28 and 33°C., favoured wilt in the former and low soil temperatures, between 17 and 29°C., favoured the disease in the latter crop.

2. It was noted that the maturity of plants also exercised some influence on their susceptibility to the pathogen, more plants of sann-hemp dying in the earlier part of the season and more of pigeon-pea succumbing to the disease in the later.

3. The simple and multiple coefficients of correlation among the three variables, soil temperatures, maturity and wilt incidence, gave significant values, indicating that the hypothesis is correct.

4. It was further noted that the values of partial coefficient of correlation between wilt incidence and soil temperature, eliminating the effect of maturity, or between wilt incidence and maturity, eliminating the effect of soil temperature, were not significant, showing that the influence of soil temperatures and maturity was a combined one on these crops.

REFERENCES

Dickson, J. G. (1923). *J. Agric. Res.* **23**, 837-870.

Fisher, R. A. (1932). 'Statistical Methods for Research Workers', Oliver and Boyd, London.

Jones, L. R.; Johnson J. and Dickson, J. G. (1926). *Wis. Agric. Expt. St. Res. Bull.* **71**, 1-144.

McRae, W. and Shaw, F. J. F. (1933). *Imp. Coun. Agric. Res. Sci. Mono, No.* **7**.

Mitra, M. (1934). *Ind. J. Agric. Sci.* **4**, 701-714.

Wallace, H. A. and Snedecor, G. W. (1930). 'Correlation and Machine Calculation'. Ames, U. S. A.

A NOTE ON THE INHERITANCE OF STERILITY IN COTTON

BY

J. B. HUTCHINSON

AND

P. D. GADKARI,

Institute of Plant Industry, Indore, Central India

(Received for publication on 8th July 1935)

In 1930 two sterile plants were observed in a crop of " Million Dollar " cotton grown by the Physiological Department of the Cotton Research Station, Trinidad, B. W. I. The seed from which the crop was grown had been supplied by the senior author, and was only two generations removed from the self-fertilised lines of the genetic plots. The strain is a form of *G. arboreum* (Linn.) var. *neglecta* (Watt) (as interpreted by Hutchinson and Ghose [1935]) and was obtained by Dr. S. C. Harland from Nanking, China, in 1923. The crop in which the sterile plants occurred had been carefully rogued for natural hybrids by the senior author at the beginning of flowering. The steriles were only observed late in the season, when they stood out owing to their continued flowering, and failure to set bolls. They exhibited no characters which would support the belief that they might be natural hybrids, and subsequent breeding experience with one of them confirms the conclusion that it was pure " Million Dollar".

The first sterile plant observed (known as Rogue A) was distinguished from normal plants by its very large flowers, and its complete sterility. All attempts to obtain seed by selfing or crossing, either as male or female, failed. An attempt was made to carry it on by grafting, and it was actually maintained for some months, but all grafted plants failed to produce monopodial buds, and developed into a series of straggling sympodia, which finally died off.

When Rogue A was discovered, a careful search for other similar plants was made. In a crop of about one acre, one other sterile plant (Rogue B) was found. This plant was in all respects similar to normal " Million Dollar " plants except that it failed to set bolls, and consequently remained vegetatively vigorous when fertile plants were declining as a result of the strain of boll production. On this plant, one boll set after self-pollination, and one after open pollination. Grafts

were made, and were maintained for some months. Gradually, however, one grafted plant after another developed the typical large flowers of Rogue A, ceased to produce monopodial buds, and degenerated into the bunch of straggling sympodia typical of the last stages of grafted Rogue A plants.

The selfed seeds obtained from Rogue B were sown in a greenhouse in Trinidad, and eleven plants were obtained. All were highly sterile, and no seed was obtained on selfing. Ten plants were vegetatively normal, and appeared to be typical of "Million Dollar" except for their sterility. One plant produced very abnormal, small, strap-shaped leaves, and very few small, deformed flowers. We are indebted to Dr. A. Skovsted for the information that the chromosome complement of this plant was $2n=26$, as in normal Asiatic cottons. Examination of pollen under a low power microscope showed that a very high proportion of the grains was shrivelled and collapsed.

Crosses were made between sterile plants of the progeny of Rogue B and normal "Million Dollar". Using Rogue B plants as female two successful crosses were obtained, one on plant 8 and one on plant 9. F_1s of ten or fifteen plants were grown, and all were completely fertile. Three F_1 plants were grown in the greenhouse (B. 8×M. D.-1, B. 9×M. D.-2 and B. 9×M. D.-3). Pollen of plants 1 and 2 was examined. On plant 1 all grains appeared to be normal, while on plant 2, 4 per cent of shrivelled grains only were observed. Backcrosses were made to normal Million Dollar, and selfed seed was collected. F_2s were grown in 1932 in Trinidad and in 1933 and 1934 at Indore, Central India. Backcross progenies were grown at Indore in 1933 and 1934. F_3s and backcross selfed progenies were grown at Indore in 1934. Since the amount of bad pollen gave an easily determined estimate of sterility, pollen counts were made on all plants. Details of percentage of shrivelled pollen in F_2, F_3, backcross, and backcross selfed are given in Table I.

TABLE I

Percentage of shrivelled pollen in crosses of Rogue B × Million Dollar

Generation	Family	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-100	Total
F_1	B. 8×M. D.-1	1	...	...	...	...	...	...	...	...	...	1
	B. 9×M. D.-2	1	...	...	...	...	...	...	...	...	...	1
F_2	B. 8×M. D.-1	36	2	...	...	...	1	3	5	5	1	53
	B. 9×M. D.-2	84	1	...	...	...	2	5	9	14	7	122
	B. 9×M. D.-3	91	8	...	1	...	2	4	8	5	4	123
F_3	B. 8×M. D.-1-84	28	7	...	...	...	...	...	...	...	...	35
	B. 9×M. D.-2-85	4	...	...	...	...	...	...	...	...	...	4
	B. 9×M. D.-2-86	4	...	...	...	...	...	...	...	...	...	4
	B. 9×M. D.-3-415	4	1	...	...	...	...	...	...	...	...	5
	B. 9×M. D.-3-412	3	...	...	...	...	...	...	...	...	1	4
	B. 9×M. D.-3-413	4	1	...	...	...	...	...	1	1	1	8
	B. 9×M. D.-3-418	5	...	...	...	...	...	1	1	2	1	10
Backcross	M. D. × (B. 9 × M. D.-2)	13	2	...	...	...	...	...	...	...	...	15
Backcross selfed	(M. D.×(B. 9×M. D.-2))-89	7	3	...	...	...	...	...	...	...	...	10
	(M. D.×(B. 9×M. D.-2))-90	11	3	...	...	...	...	1	...	2	2	19

The F_2 progeny fell into two distinct groups. In one group most of the pollen produced was bad and no seed was set either on selfing or by open pollination, while in the other group nearly all the pollen was good and the plants gave a normal crop of seed cotton.

The F_2 data may be summarised as follows:—

Family	Fertile	Sterile	Total
B. 8×M. D.-1	38	15	53
B. 9×M. D.-2	85	37	122
B. 9×M. D.-3	99	24	123
Total (observed)	222	76	298
(On 3 : 1 basis)	223·5	74·5	298·0

The plants of the backcross were all normal, giving a low percentage of bad pollen and a normal crop of seed cotton. Two families were grown from selfed seed of backcross plants. One of them gave all fertile normal plants while the other gave fourteen fertile, with little bad pollen, and five sterile with 60-100 per cent bad pollen.

Seven small F_3 families were grown. Of these, four gave only fertile plants with little bad pollen, and three segregated, giving in all 13 fertile : 9 sterile plants.

The behaviour of all families agrees well with expectation on the basis of a single-factor difference between fertility and sterility, with fertility dominant. Sterility was equal on male and female sides, but was not quite complete. It thus appears to resemble in genetic behaviour Beadle's [1930] case of a gene for asynapsis in maize. The original sterile Rogue B gave one somatically abnormal plant in a progeny of eleven, the others being somatically normal. This suggests that the disturbance to gamete formation responsible for sterility has other effects also which may be manifested among what few progeny are obtainable by selfing. The somatic change which occurred in grafts of the original Rogue B, whereby they developed the characteristics of the apparently completely sterile Rogue A, are of considerable interest, but investigation of the phenomenon depends upon the success of attempts to induce similar changes in sterile segregates from heterozygous lines now under cultivation.

Acknowledgments

The investigation here reported was commenced while the senior author was Assistant Geneticist at the Empire Cotton Growing Corporation's Cotton Research Station, Trinidad, B. W. I. He has pleasure in acknowledging his indebtedness to the Geneticist, Dr. S. C. Harland, under whom he was working, and to the Physiologist, Dr. T. G. Mason, who made available the original sterile plants.

The authors completed the work at the Institute of Plant Industry, Indore, Central India, during the tenure by the junior author of a research scholarship from the King Edward Memorial Society, Central Provinces and Berar, to which Society he takes this opportunity of tendering his thanks.

REFERENCES

Beadle, G. W. (1930). *Cornell Agric. Expt. Stn. Mem.* **129**, 1.

Hutchinson, J. B. and Ghose, R. L. M. (1935). *In Press.*

A NOTE ON A SURVEY OF THE DISEASE OF MALFORMATION IN THE PUNJAB-AMERICAN COTTONS

BY

MOHAMMAD AFZAL, B.Sc. (AGRI.), A.I.C.T.A.,
Cotton Research Botanist, Punjab, Lyallpur,

SANTOKH SINGH JAGGI, B. Sc. (AGRI.),
Senior Assistant,

AND

BISHAN SINGH, B.Sc. (AGRI.),
Junior Assistant

(Received for publication on 23rd July 1935)

INTRODUCTORY

For the past few years the American cottons in Lyallpur have been afflicted with a peculiar growth-abnormality. The chief symptoms of the disease have been described elsewhere [Afzal, 1933] and will only be briefly reviewed here.

The chief feature which strikes the eye is the diminution of all plant organs ; the height of the plant, size of internodes, leaves, floral organs and the bolls are very much reduced in size. In addition, the leaves bear streaks of light yellow colour and appear mottled. There is fairly profuse flower-bud formation, but the shedding is likewise high and in extreme cases leads to sterility. The bolls which are formed give but few viable seeds. The attack may either be confined to a few limbs or the entire plant may be affected.

The disease was first noticed at Lyallpur on a few plants of American cotton in 1929, but continued to spread in later years and came to be reckoned as a serious disease of cotton. Up to 1933 it was only known to attack American varieties of cotton, but during this year one plant of *G. indicum* at the Cotton Research Station, Lyallpur, and two plants of *Mollisoni* at the Agricultural Farm, Sirsa, were also attacked. Since then many more plants of *Desi* cotton have been attacked.

Since its first appearance in 1929, the disease has continued to spread year after year and became a menace to cotton-growing in the Punjab Agricultural College Estate.

Diseases of cotton of a similar nature have been reported from the United States of America [Cook ; 1913, 1924, 1927], West Indies [Nowell, 1914], China and Haiti [Cook, 1920, 1923] and Southern India [Kottur and Patel, 1920]. Of late years the disease has attained serious proportions in parts of Bombay Presidency. From all outward appearances the disease in Bombay appears to be very similar to that present in the Punjab, but in Bombay it attacks the indigenous varieties of cottons, while in the Punjab the attack is mostly confined to American cottons. A careful study of the diseased plants has shown that the disease found in the Punjab is very similar to " Stenosis " or " Smalling " described by Cook [1924] and the same name has, therefore, been tentatively given to the disease.

Extent of damage

As has been said above, the extent of the damage caused by the disease at the Punjab Agricultural College Estate has increased every year since its incipient appearance in 1929. Counts made in 1932 showed that a little over 40 per cent of the plants were attacked in certain fields. During 1933 an attempt was made to collect data regarding the occurrence of this disease in other parts of the Province, but on account of the lack of staff much headway could not be made. The Indian Central Cotton Committee were, therefore, requested to provide funds so that a thorough survey of the incidence of the disease could be made throughout the cotton-growing tracts of the province. This was undertaken in 1934 and the present note mainly embodies the results.

Plan of work

A village-to-village enquiry was made in all the important cotton-growing tracts of the province and information collected regarding the prevalence of the disease, its time of appearance, etc. In all 138 villages were visited, but the disease was recorded from eight only, for which detailed data are given in Table I. It will be noticed that the disease was present only in a few places in Lyallpur, Multan, Lahore and Sargodha districts. It was most severe in Lyallpur, while only occasional plants were met with in the other three districts. The rest of the villages visited were found to be free from this disease.

TABLE I

Results of survey of the disease of malformation in the Punjab-American cottons in different districts of the cotton belt

Names of districts	No. of villages surveyed	Area examined (acres)			Total	Percentage of smalling	Remarks
		Desi	American	Desi and American mixture			
Lyallpur . .	28	15	659	..	674	1·014	Includes the Punjab Agricultural College Estate, where the attack was 12·9 per cent and in some seriously attacked patches it was as much as 40 per cent.
Sheikhupura . .	10	4	140	29	173	*Nil*	
Gujranwala . .	4	..	26	27	53	*Nil*	
Jullundur . .	3	19	..	..	19	*Nil*	
Hissar . . .	7	128	3	..	131	*Nil*	
Gujrat . . .	7	..	111	..	111	*Nil*	
Jhang . . .	12	..	235	..	235	*Nil*	

Shahpur . .	19	..	307	..	307	1·23	Only in one locality and in one particular field the attack was up to 3·7 per cent while affected plants could be seen scattered in this field.
Montgomery . .	15	7	364	..	371	*Nil*	
Multan . .	11	29	277	..	306	5·6	Only in a ½ acre field.
Lahore . . .	2	..	35	..	35	..	Only 4 plants affected in one field.
Amritsar . .	6	72	..	..	72	*Nil*	
Ferozepur . .	2	10	1	..	11	*Nil*	
Karnal . . .	3	25	..	..	25	*Nil*	
Gurgaon . .	5	45	..	..	45	*Nil*	
Ambala . .	2	18	..	..	18	*Nil*	
Ludhiana . .	2	12	..	..	12	*Nil*	

Detailed records were taken at the Cotton Research Station, Lyallpur, and it was found that the attack starts in July and increases progressively till the middle of September, after which there is no further increase. Figures appear in Table II.

TABLE II

Percentage of attack at different periods of growth

1932		1934	
Date of observation	Percentage of attack	Date of observation	Percentage of attack
1st July . . .	1·7	13th July . . .	3·87
1st August . . .	16·3	2nd August . .	5·48
1st September . .	42·5	17th August . .	10·09
		3rd September . .	15·03
		15th September .	16·40

A certain degree of varietal preferences is also exhibited by the disease as the following figures will indicate.

TABLE III

Percentage of attack on different varieties

Name of variety	Percentage of attack
4 F	10·58
43 F	2·75
289 F	17·46

Although the diseased plants are scattered about irregularly in any one field, the severity of the disease varies with different localities. The subjoined figures from fields situated in different parts of the Agricultural Research Farm, Lyallpur, show this point very clearly.

TABLE IV

Percentage of attack in different fields

Variety	Square No.	Percentage of attack	Remarks.
L. S. S. . .	27	20·26	Rich land, well manured and intensively cultivated
38 F . . .	27	20·16	
45 F . . .	27	20·61	
L. S. S. . .	32	3·02	Light land
38 F . . .	32	4·92	
45 F . . .	32	4·92	

The above figures are, in a way, rather remarkable. The locality in which the crop is grown seems to have a preponderating effect, while the varietal differences in the severity of attack sink into insignificance.

NATURE OF THE DISEASE

Although similar diseases of cotton have been extensively reported from various parts of the world, their exact nature has not so far been discovered.

Likhite and Desai [1935] have discovered an excessive accumulation of starch in the stenosised organs but this accumulation of starch appears to be the result rather than the cause of the disease.

A few preliminary experiments to find out the nature of the disease were performed and are briefly described below :—

(*a*) *Virus nature*

In order to ascertain if the disease was of virus nature the following experiments were performed :—

(*i*) *Juice inoculation.*—Ten plants of each of 4F, 289F and 43F were inoculated with an extract of diseased plants at different ages. Thus, in all 119 plants were inoculated but none of them showed any sign of disease.

(*ii*) *Grafting.*—Both the touch method and the bottle-grafting method described by Harland were used on several plants and no transmission of the disease from the diseased to the healthy branch was noticed.

(*b*) *Possible hereditary nature*

Self-bred seed procured from the small-sized bolls borne on plants affected by smalling was sown in 1930. The resulting crop was absolutely normal in all ways and showed no attack of disease. The same experiment was repeated during 1934, the progeny of the plants showing no sign of disease. This goes to show that the disease is not of a hereditary nature.

(*c*) *Relation of disease to soil conditions*

Different fields of the Agricultural Research Farm, Lyallpur, have, this year, shown varying degrees of attack. This points to some type of relationship between the kind of soil and the severity of the disease. More figures are, however, required to thoroughly establish this point.

Other plants attacked by "smalling" or a disease very similar to it

An effort was made to find alternative host plants, if any, and it was found that the following crop plants were attacked by a disease which in outward appearance is very similar to the "smalling" of cotton :—

Lobia (*Dolichos Lablab*) . . .	A very large number of plants found in cotton fields at Lyallpur.
Mung (*Phaseolus Mung*) . . .	Found in a village near Jaranwala in the Lyallpur district.
Mash (*Phaseolus radiatus*) . . .	
Chillies (*Capsicum annuum*) . .	Found in several plots in the Lyallpur and Ludhiana districts.

It is suggested that an exhaustive study of the alternative host plant is likely to throw a great deal of light on the modes of perennation of this disease. This work is now in progress.

Conclusions

The following general conclusions could be drawn :—

(*i*) The disease is serious in the Punjab Agricultural College Estate and neighbouring fields, while it is really of no consequence in the other parts of the cotton belt of the Punjab.

(*ii*) The disease primarily attacks the American varieties of cotton, while very few plants of the indigenous varieties are attacked.

(*iii*) The attack starts when the plants are about a month old. The disease is very active during August and early part of September.

(*iv*) The plants which are attacked while still young, remain very much stunted in size and bear either none or very few small bolls.

(*v*) The attack spreads upwards in the plant from the incipient focus in a fairly regular way, but sometimes a few limbs escape the effects of the disease.

(*vi*) The disease is not of a hereditary nature. It is, however, not definitely known whether it is physiological in character or a virus. Its relationship with soil conditions is likewise obscure.

(*vii*) Certain other crop plants showing symptoms similar to those of cotton have been found.

Acknowledgments

This work was carried out with the help of the staff and funds provided by the Indian Central Cotton Committee.

REFERENCES

Afzal, M. (1933). *Ind. J. Agri. Sci.* **3**, 933.

Cook, O. F. (1913). *U. S. Dept. Agric. Bur. Plant Ind. Cir.* **120**.

———— (1920). *J. Hered.* **11**, 99—110.

———— (1923). *Ibid* **14**, 323—335.

———— (1924). *J. Agric. Res.* **28**, No. 8.

———— (1927). *U. S. Dept. Agric. Bur. Plant Ind. Bull.* **1484**.

Kottur, G. L. and Patel, M. L. (1920). *Agric. J. Ind.* **15**, 640.

Likhite, V. N. and Desai, G. L. (1935). *Cur. Sci.* **3**, No. 8.

Nowell, W. (1914). *West Ind. Bull.* **14**, No. 4.

INVESTIGATIONS ON THE WOUND-PARASITISM OF CERTAIN *FUSARIA*

BY

ANIL MITRA,

Department of Botany, University of Allahabad

(Received for publication on 12th November 1934)

(With Plate XXIX)

I. Introduction

Fusaria were regarded by de Bary as nothing more than obligate saprophytes. Later a large number of investigators reported the association of these fungi with various diseases of field and garden crops, but it was Smith [1899] who first demonstrated by experiments the exact relation of *Fusarium* to wilts of cotton, water-melon and cow-pea. Duggar [1909] wrote "It is apparent that the old view which held species of the genus *Fusarium* to be largely saprophytic, must be considerably modified" and Stevens [1925] has recently remarked, "Taken as a whole the genus is one of the most injurious with which plant pathology has to do". In India the more well-known cases of their parasitic activities are the wilts of economically important plants, such as *Cajanus indicus*, *Cicer arietinum*, *Gossypium* sp. and the rots of potatoes.

Most of the *Fusaria* collected by the writer were growing saprophytically. In view of the above remarks it was thought that an interesting result would be obtained if some of them at least showed parasitic activities. The present paper gives an account of the artificial inoculation experiments carried out on apples and potatoes with six species of *Fusarium*.

II. Source of the *Fusaria*

1. *F. camptoceras* Wr. et Rkg.—From the ear of *bajra* (*Pennisetum typhoideum*).

2. *F. incarnatum* (Rob.) Sacc. re-named as *F. semitectum* var. *majus*. Wr.—From a darkened young shoot of *Aegle marmelos*.

3. *F. semitectum* Berk. et Rav.—From greyish spots on a green fruit of *Citrus medica*.

4. *F. diversisporum* Sherb.—From the petiole of a wilted leaf of *Carica papaya*.

5. *F. viride* (Lechm.) Wr. re-named as *F. solani* var. *medium* Wr.—From the base of the stem of a wilted *Cajanus indicus*.

6. *F. moniliforme* Sheldon.—From dead leaf sheath of *Andropogon Sorghum*.

The writer is greatly indebted to Dr. Wollenweber for identification of the species.

Bacteria-free single spore cultures grown on Brown's medium were the sources of the inocula.

III. Inoculation of apples

The apples used were of two varieties namely the "hill" apple and the "Kashmir" apple*. They were specially selected to ensure, as far as possible, apples of the same quality and age. They were washed thoroughly in water, wiped dry and then swabbed lightly with alcohol. Inoculations were made by the method devised by Granger and Horne [1924] and used by Mitter and Tandon [1929]. Briefly the process may be described as follows.

A sterile cork-borer was thrust into the apple. It was then taken out along with the plug of the tissue of the apple and suspended over mercuric chloride solution to prevent contamination. The inoculum was placed in the cavity and the plug was replaced by pushing with a sterile glass rod passing through the cork-borer. It was then sealed with a melted mixture of paraffin and beeswax led around the margin of the plug. The inoculated apple was then rubbed lightly with alcohol and wrapped in sterile paper. A minor change, which proved useful, consisted in using a straight needle for inoculations in place of the usual hooked one. Five apples of each variety were inoculated with one species *Fusarium* and therefore 60 inoculations were made. Ten apples, five of each variety, similarly treated but not inoculated, were kept as controls. The apples were left undisturbed for 35 days at room temperature (19·5°-22·8° C.). The rots were measured by weighing the apples first, scraping out the rotted tissue very carefully and then weighing a second time. The difference between the two weighings gave the amount of rotted tissue and from the figures the percentage of damage was calculated.

* The "Kashmir" apple differs from the other in being elongated in the vertical or blossom axis, sweeter, less acidic and brighter in colour.

TABLE I

Showing the result of inoculation of apples with F. moniliforme *and* F. viride

Fungus	Variety of apple used	No. of apples inoculated	No. of apples infected	Mean diameter of diseased area (cm.)	Mean percentage of rot
F. moniliforme .	"Hill" .	5	4*	3·8 × 3·6	22·32
	"Kashmir".	5	5	3·7 × 3·4	21·7
F. viride . .	"Hill" .	5	5	1·9 × 1·7	1·52
	"Kashmir".	5	5	1·5 × 1·4	1·36

*Rot of the fifth apple was due to *Penicillium* as well as *Fusarium*.

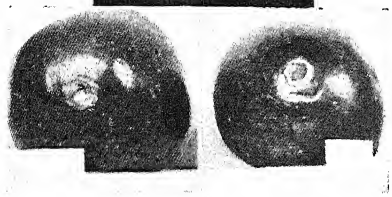
Both the varieties of apples inoculated with *F. moniliforme* (Plate XXIX, figs. 3, 4) showed an average damage of 22·01 per cent. Those inoculated with *F. viride* (Plate XXIX, fig. 2) showed on an average only 1·44 per cent damage. Apples inoculated with *F. camptoceras*, *F. semitectum* and *F. incarnatum* and the controls remained perfectly sound and showed no signs of rot or decay (Plate XXIX). That the rots measured were due to the *Fusaria* with which apples were inoculated was proved by the isolation of the respective *Fusaria*, unmixed with other fungi, from aseptic blocks cut from the diseased part of each apple just before scraping. Only in the case of *F. moniliforme* the fifth apple of the "hill" variety showed a much greater rot and from it *Penicillum* mixed with *Fusarium* was isolated. This apple was not included in the estimation of the rot. The above experiment has been repeated with similar results, *F. moniliforme* showing a decided wound-parasitism on both the varieties of apples. Unfortunately in the second experiment no apples were inoculated with *F. viride* and so the results obtained in the previous experiment with this fungus could not be verified.

The writer has not come across any published record of the occurrence of *Fusarium* on apples in India. He is also not aware of any record of *F. moniliforme* as showing any kind of parasitic activity on apples in India.

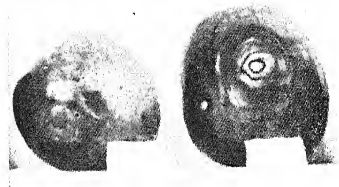
IV. INOCULATION OF POTATOES

Granger and Horne [1924] point out that the method of inoculation used for apples is not applicable to potatoes, for the successful removal of a plug depends on the friction between the tissue of the thing to be inoculated and the surface of the cork-borer. The tissue of potato being soapy it is almost impossible to

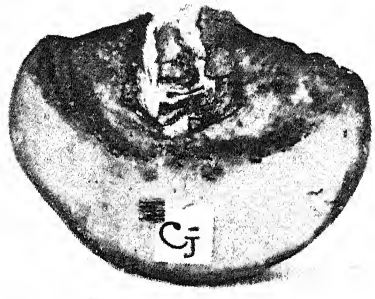
1 2



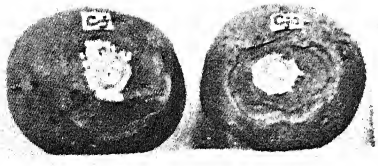
3

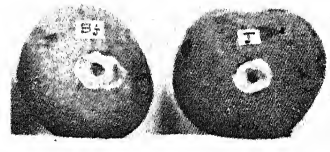


4



5





MITRA-PHOTO

6 7 8 9

1. "Hill" apple used as control, showing no rot. 2. "Hill" apple showing the slight rot caused by *F. viride*. 3. "Hill" apples showing the rot due to *F. moniliforme*. 4. "Kashmir" apples showing the rot caused by *F. moniliforme*. 5. Half of a potato showing the nature of the dry rot caused by *F. viride* (*Cj.*). 6. Entire potato showing the dry rot caused by *F. viride* (*Cj.*). 7. Entire potato showing the dry rot caused by the saltant No. FV_3. of *F. viride* (*CjS.*) 8. Potato inoculated with *F. camptoceras* (*Bj.*) showing no rot. 9. Potato inoculated with *F. moniliforme* (*J*) showing no rot.

remove an undamaged plug. For this reason the following method was adopted. The surface of a previously washed potato was swabbed lightly with alcohol. A sterile cork-borer was inserted into it for some distance. It was then taken out and the plug that remained attached to the tissue of the potato was cut half way down by the tip of a sterile pointed scalpel which was inserted obliquely at any point in the circular cut made by the borer. The detached plug was lifted up by the pointed end of the scalpel inserted in it a little sideways and the inoculum was put into the cavity. The plug was replaced and sealed with a melted mixture of paraffin and beeswax. As usual it was then wiped with alcohol and wrapped in sterile greased tissue paper. That this method is quite satisfactory is proved by the fact that out of more than fifty potatoes that were inoculated not one showed any rot due to foreign infection. Six potatoes were inoculated with a single species of *Fusarium* and six similarly treated but not inoculated, were kept as controls. They were left undisturbed for 24 days at room temperature (20°-25° C.). In this case no attempt was made to determine the amount of rot.

Result :*—

Potatoes inoculated with *F. incarnatum*—undamaged.

Potatoes inoculated with *F. diversisporum*—undamaged.

Potatoes inoculated with *F. semitectum*—undamaged.

Potatoes inoculated with *F. semitectum* saltant—FS_1. (This saltant originated as a sector in a Petri dish culture during a cultural study of the parent. It has been described by Mitra [1934,1])—undamaged.

Potatoes inoculated with *F. camptoceras*—undamaged (Plate XXIX, fig. 8).

Potatoes inoculated with *F. moniliforme*—undamaged (Plate XXIX, fig. 9).

Potatoes inoculated with *F. viride*—all showed heavy infection, about half of them being rotten (Plate XXIX, fig. 6).

Potatoes inoculated with *F. viride* saltant—FV_3. (Origin and character given in Mitra [1934,1]; showed the same damage as the parent.) (Plate XXIX, fig. 7).

Potatoes used as controls—all remained healthy and showed no wrinkles.

The rots caused by *F. viride* and its saltant were dry rots, the nature of which is shown in Plate XXIX, figs. 5, 6, 7. The rotted potatoes showed a wrinkled, sunken patch round the plug and whitish pustules were observed to come out on the surface near the plug. *F. viride* and its saltant were isolated in a pure form both from the aseptic blocks cut from the diseased parts of the potatoes and from the whitish pustules on the surface.

---

* A preliminary report on the parasitism of *F. viride* was published as a note entitled " A new wound-parasite of potato tubers " in *Nature* **133**, page 67 : January 13, 1934.

In answer to an enquiry, Mr. S. F. Ashby of the Imperial Mycological Institute, Kew, informs the writer that he has not been able to find a record of *F. viride* on potato nor of any tests of its parasitism on that host. Recently Wollenweber [1931] has re-named this fungus as *F. solani* var. *medium* Wr. but its pathogenicity does not appear to have been tested. Out of the many investigators who have reported the occurrence of *F. solani* (Mart.) App. et Wr. on rotten potatoes only Kasai [1920] maintains that he obtained positive results with artificial inoculations. This species according to Schmidt [1928] is a saprophyte very liable to confusion with the closely related *F. caeruleum* which is known to be a virulent parasite of potatoes and according to Wollenweber [1923] is a " fast spreading inhabitant of rotting potatoes ".

V. Conclusions

From the foregoing experiments it is seen that some of the species of *Fusarium* are restricted in their capability to parasitise different hosts. *F. camptoceras* apparently growing parasitically on *Pennisetum typhoideum* could not infect either apples or potatoes. *F. viride* could infect potatoes heavily, when wounded artificially, but showed a very weak and doubtful parasitism on apples. *F. moniliforme*, on the other hand, heavily infected apples but was unable to parasitise potatoes. The saltants did not differ from their parents in their parasitic activities on the potato. The saltant of *F. viride* was as virulent as its parent while both *F. semitectum* and its saltant lacked parasitic power. Working with *Fusarium* Mitter [1929] found that the saltants tested exhibited less virulence on apples than their respective parents.

Fusarium diversisporum has been shown by Sherbakoff [1915] to be a rot producer of potatoes but under the conditions of the present experiment this organism was found to be quite incapable of producing a rot.

The *Fusaria* that showed parasitic activity were mostly capable of growing saprophytically. Of such forms *F. moniliforme* is also recorded to cause corn mold disease in America by Leonian [1932]. This confirms the general experience that at least some of the *Fusaria* growing saprophytically in nature are not obligate saprophytes. Some of them under certain circumstances are able to show parasitic activity and it is possible that if they cannot infect healthy fruits and tubers, they may still cause their rots by getting into the tissues through accidental wounds.

VI. Acknowledgment

The writer wishes to acknowledge his indebtedness to Professor J. H. Mitter for suggesting the problem and for helpful guidance.

Summary

1. Six species of *Fusarium, viz., F. camptoceras, F. viride, F. diversisporum, F. incarnatum, F. semitectum* and *F. moniliforme* were tested as to their parasitic activities on apples and potatoes by artificial inoculation.

2. *F. moniliforme* was found to be able to cause a great amount of rot in both the " Kashmir " and " hill " varieties of apples.

3. Potatoes were attacked only by *F. viride* (*F. solani* var. *medium* Wr.) which caused a dry rot.

4. Saltants tested exhibited the same parasitic activities on potatoes as their respective parents.

5. As the *Fusaria* that showed parasitic activities were growing as saprophytes, it is seen, that if they cannot infect healthy fruits and tubers, they may still cause a rot by getting into their tissues through accidental wounds.

6. So far as the knowledge of the writer goes there is no published record about *F. moniliforme* and *F. viride* (*F. solani* var. *medium*) as showing wound-parasitism on apples and potatoes respectively.

REFERENCES

Duggar, B. M. (1909). 'Fungous Diseases of Plants.'

Granger, K. and Horne, A. S. (1924). *Ann. Bot.* **38**, 212.

Kasai, Mikio (1920). *Ber. Ohara—Inst. Japan* **1**, 519. (Abs. in *Rev. Appl. Myc.* **1**, 80 ; 1922).

Leonian, L. H. (1932). *Agric. Expt. Stn. West Verginia Univ. Bull.* **248**.

Mitra, Anil (1934,1). *J. Ind. Bot. Soc.* **13**, 255-268.

——— (1934,2). *Nature* **133**, 67.

——— (1934,3). *Current Sci.* **3**, 202.

Mitter, J. H. (1929). *Ann. Bot.* **43**, 379.

Mitter, J. H. and Tandon, R. N. (1929). *J. Ind. Bot. Soc.* **8**, 212.

Schmidt, E. (1928). *Arb. Biol. Reichsanstalt.* **15**, 537-592. (Abs. in *Rev. Appl. Myc.* **7**, 466 ; 1928).

Sherbakoff, C. D. (1915). *Cornell Univ. Agric. Expt. Stn. Mem.* **6**.

Smith, E. F. (1899). *U. S. Dept. Agric. Bull.* **17**.

Stevens, F. L. (1926). ' Plant Disease Fungi.'

Wollenweber, H. W. (1923). Saurer's 'Handbuch der Pflanzenkrankheiten' Band III.

———————(1931). Fusarim Monographie.

STUDIES ON *PENNISETUM TYPHOIDES* (BURM.) STAPF. AND HUBBARD [SYN. *P. TYPHOIDEUM* (RICH)]—THE PEARL MILLET, PART II

SPIKELET-BEARING BRISTLES*

BY

G. N. RANGASWAMI AYYANGAR, B.A., I.A.S.,

Millets Specialist, Agricultural Research Institute,

V. GOMATHINAYAGAM PILLAI, B.Sc. (Ag.).,

AND

P. V. HARIHARAN, B.Sc. (Hons.),

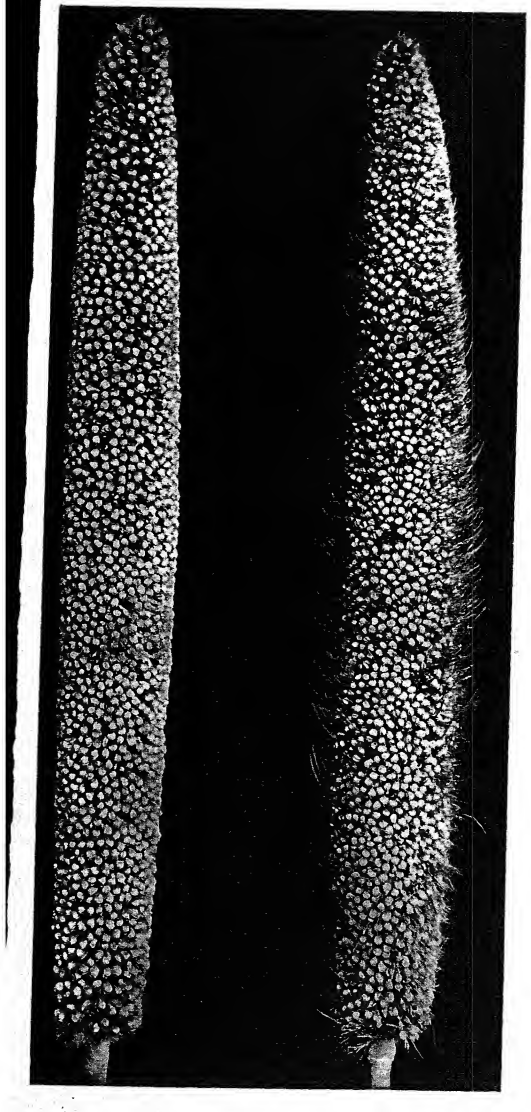
Assistants, Millets Breeding Station, Coimbatore

(Received for publication on 5th February 1935)

(With Plate XXX)

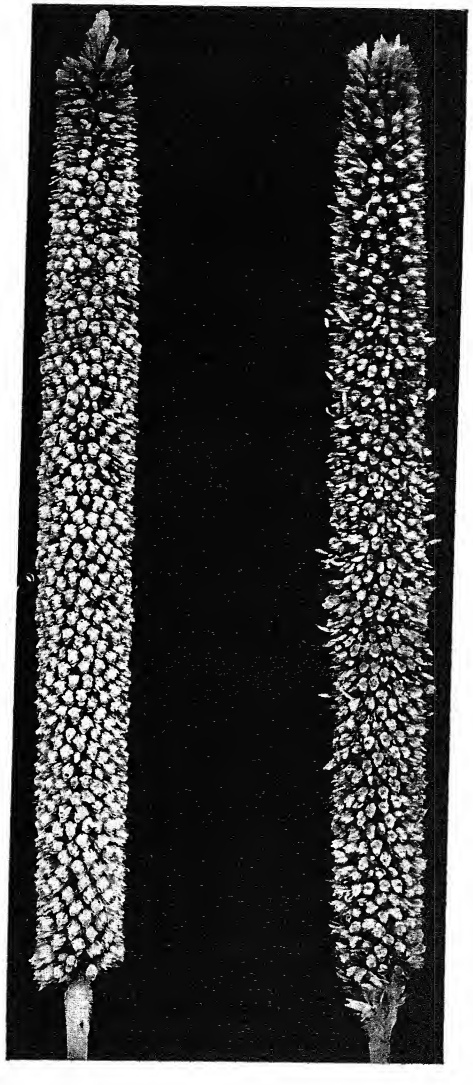
Pennisetum typhoides (Burm.) Stapf. and Hubbard [Syn. *P. typhoideum* Rich] is the pearl millet, '*bajra*' of North India and '*cumbu*' of Madras. The earhead of this millet is spike-like, being composed of a central rachis and a number of seriately disposed, closely-packed fascicles. Each fascicle includes one or more spikelets and a whorl of free bristles. A single spikelet is really a secondary spike having an upper and a lower floret, the former being hermaphrodite and the latter staminate, sterile or barren. Surrounding the spikelets in each fascicle there are 30-70 bristles (average 40). The outer bristles are shorter than the inner. In most cultivated varieties these bristles are not prominent. Godbole [1927] notes that the number of these bristles decreases as the number of spikelets within the whorl increases. Rare instances have been met with in which the longer individuals in this bristle group elongate much beyond the grain surface of the spike and give the earhead the hairy look associated with some of the races of the Italian (fox-tail) millet (Plate XXX, fig. 1). The occurrence and inheritance of these bristles in the Italian millet has been worked out [Rangaswami Ayyangar *et al.*; 1933,1]. The fact that some of these bristles in the Italian millet bear spikelets at their ends has been noted and the inheritance of this peculiarity studied [Rangaswami Ayyangar *et al.*; 1933,2]. In

* Paper read before the Agricultural Section of the 22nd Session of the Indian Science Congress, January 1935



Ordinary. Bristled.

FIG. 1.



Ordinary. Spikelet-bearing bristled

FIG. 2.

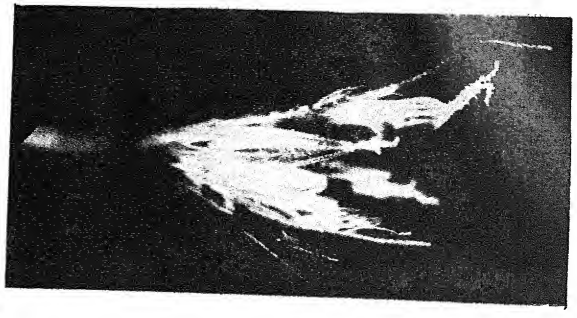


FIG. 3. Flower-bearing bristle with protruding stigma.

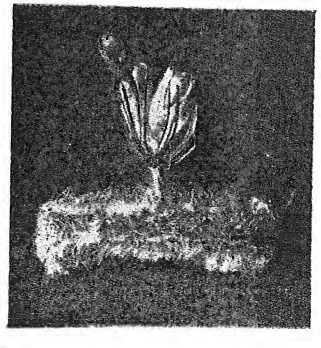


FIG. 4. Grain-bearing bristle.

some African races of the pearl millet recently received at Coimbatore, a tendency to shedding of spikelet whorls was noticed and it would appear that this disability must have militated against the perpetuation of many long-bristled types as economic varieties. Evidently the spike-like disposition of the grains is inconsistent with the accommodation of obtrusive bristles. That the reduction in bristles, both in number and in length, has kept pace generally with the packing of the grains on the earhead, will be obvious from *P. leonis* (Stapf. and Hubbard) which has the densest ear and the shortest bristles.

In the year 1928 certain bristled earheads which occurred in a collection from the Bellary District of the Madras Presidency were sown at the Millets Breeding Station. In five of the plants raised, odd bristles were noted to have floral parts attached to their ends. These were first suspected to be dry anther sacs sticking on to the bristles, but later on were found to be definite out-growths from the latter (Plate XXX, fig. 2). These bristles were obviously continuations of the fascicle axis and bore the spikelets at the ends as lateral appendages. Beyond these appendages, the bristle got prolonged, but by the shedding of the prolongation at maturity the spikelet appeared as the apparent end of the bristle (Plate XXX, figs. 3 and 4). The occurrence of the spikelet-bearing bristles was however noted only in comparatively short-bristled earheads. Seeds from such earheads were sown and in the year 1930, out of 337 plants thirty-one showed these abnormalities. Two of these earheads were selfed and in 1931 when these were sown, forty-nine out of 166 manifested this abnormality. The increase in number is not surprising in the selfed material of this protogynous crop. Two heads were again selfed and in the 1932 crop ninety-eight out of 200 plants had earheads showing stray bristle-bearing spikelets. A detailed analysis of this population was made in that year. It was noticed that there was a total of 673 earheads and of these 309 had spikelet-bearing bristles. In these 309 heads, the number of such spikelet-bearing bristles was on an average twenty, with a range from one to sixty per head, though in odd cases it went up to a hundred. Of these spikelet-bearing bristles all the spikelets at the ends were examined and it was noticed that only 166 bore grains out of a total of nearly 3,500 spikelets. These grains had eighty-eight per cent of germination while the ordinary grains had ninety-five per cent. They weighed half the weight of an equal number of normal grains.

These results show that the more prominent of these bristles are the prolongation of the fascicle axis with stray tendencies for developing lateral spikelet appendages a little below the apex. That they could have normal spikelets in rare instance has been shown above. The reference of Hofmeister to bristles bearing glumes is not clear. If there were indications of these glumes, a wider search would have led to the detection of at least weak spikelets on some of the bristles. Arber [1931] does not favour Hofmeister's view but is inclined to regard the ultimate bristle-shoots as equivalent to spikelets. The

existence of these spikelets with their usual complements of hermaphrodite and sterile flowers on the more prominent of these bristles, leads one to conclude that these grain-bearing bristles are prolongations of the fascicle axis. This phenomenon will, we expect, throw fresh light in the interpretation of histological data through which the origin of these structures has till now been sought to be pursued.

These spikelet-bearing bristles have also occurred in other imported varieties both African and Indian. Their occurrence in their new habitat may add weight to isolated observations in respect of other latent characters and may be considered as part of the reactions consequent on new place effect.

Summary

In *Pennisetum typhoides* (Stapf. and Hubbard)—the pearl millet, spikelet-bearing bristles have been met with. Their examination sets down the bristle as the prolongation of the fascicle axis. The spikelet is borne as lateral appendage, near the end of this prolongation.

REFERENCES

Arber, A. (1931). *Ann. Bot.* **45**, 401.

Godbole, S. V. (1927). *Mem. Dept. Agric. Ind., Bot. Ser.* **14**, 247.

Rangaswami Ayyangar, G. N.; Narayanan, T. R. and Narayana Rao, T. (1933,1). *Ind. J. Agric. Sci.* **3**, 207.

——————————— (1933,2). *Ind. J. Agric. Sci.* **3**, 552.

ABSTRACT

The Storage of Avocado Pears. C. W. WARDLAW and E. R. LEONARD.

(*Mem. No. 1 of the Low Temperature Research Station of the Imperial College of Tropical Agriculture, Trinidad, British West Indies.*)

The following are extracts from the above paper :—

INTRODUCTION

In preliminary studies relating to the storage behaviour of West Indian avocado pears (1), it was found that the majority of local " varieties" were unsuitable for refrigerated transport overseas because of their tendency to chill at temperatures insufficiently low to retard maturation processes. The fact that a few " varieties " were sufficiently cold-resistant, however, indicated that further storage trials might be undertaken with useful practical results. Accordingly, during the avocado season of 1934, a comprehensive series of storage experiments was again carried out, the results of which are summarised below. With a view to elucidating peculiarities in the physiological behaviour of this fruit during ripening, investigations into the nature of respiration, chilling, tolerance of gas-storage and relevant biochemical aspects have also been commenced.

GENERAL OBSERVATIONS

Anticipating, for the sake of greater clarity, observations and conclusions substantiated in later sections, some general characteristics of West Indian avocado pears may be briefly mentioned at this point.

1. With very occassional exceptions (*e.g.*, the *Pollock* variety), West Indian avocados are of seedling origin and show a striking range in variation in a number of important characteristics :

(*a*) shape may vary from small sub-spherical fruits to elongated bottle-necked fruits of large size ;

(*b*) colours include dark green, pale green, yellowish green, and purple ;

(*c*) the skin may be thick or thin, waxy or dull, rugose or smooth, relatively soft in texture or distinctly woody ;

(*d*) flesh colour varies from a medium yellow to pale greenish-white ;

(*e*) leaving aside questions of palatability which are difficult to define, but which may range from an insipid, floury to a rich nutty flavour, the flesh may vary in the matter of oil-content, development of fibre and thickness ;

(1) " Preliminary Observations on the Storage of Avocado Pears ", by C. W. Wardlaw, *Tropical Agriculture*, XI, No. 2, pages 27—35, 1934.

(*f*) variations are also readily apparent in the size and shape of the stone its proportion to the total weight of the fruit, and the nature of its insertion, *i.e.*, loose or adherent to the flesh.

2. During cold storage, even at relatively low temperatures, maturation processes slowly continue so that fruits ultimately become ripe and finally over-ripe. In the few varieties which are sufficiently cold-resistant or tolerant of low temperatures, such ripening apparently takes place in a more or less normal manner, the flesh at maturity being of good flavour and free from internal breakdown, discolouration or other obvious abnormalities. On the other hand, in the majority of varieties, *i.e.*, those which do not possess adequate cold-resistance, maturation processes also continue but it is evident that the metabolic trend is abnormal. Thus, although such fruits eventually become soft in cold storage, inspection of the flesh at once shows that chill effects have been induced. The range in genetical constitution outlined above is strikingly exemplified in the matter of cold-resistance : this extends from varieties subject to chilling after 15 days at 53°F. to those which ripen normally during 40 days at 40°F.

3. A comparable though not necessarily parallel variation in response to gas-storage conditions has also been observed : this ranges from fruits which mature normally after treatment to atmospheres containing sub-normal oxygen or high carbon dioxide concentrations, to those in which pronounced pathological symptoms are induced by such treatments.

4. Considerable differences are also apparent in the rates of ripening of different varieties under controlled conditions and in their subsequent keeping quality.

5. Variations in genetical constitution are reflected in the resistance of different varieties to anthracnose and to other storage diseases : this ranges from fruits which are severely affected in the field prior to reaping to those in which little wastage develops even after prolonged storage. So far susceptibility to fungal pathogens has not been correlated with any particular type of rind.

6. In general, the collective genetical characters of West Indian avocado pears and the impress of climate and environment are such that the behaviour of varieties in cold storage shows marked departures from the records obtained by investigators elsewhere.

* * * * * * * *

Summary and Recommendations

1. An account is given of further investigations into the cold storage behaviour of West Indian avocado pears, with a view to preparing the way for an export industry to Canadian and British markets.

2. West Indian avocado pears show a striking range in variation in colour, shape, size, texture of skin, proportion of stone to pulp, colour and fibre-content of pulp and in palatability. When fruits are placed in cold storage this genetic variability is further reflected in differences in keeping quality, susceptibility to fungal pathogens, rate of ripening and to tolerance of low temperatures.

3. In confirmation of earlier investigations, it was again found that a large majority of Trinidad varieties do not possess the keeping quality and are not sufficiently cold-resistant to be held for normal commercial periods (16-25 days) at 45°F. At this temperature such varieties are subject to chilling, with concomitant discolouration of the pulp and in some instances of the skin. On the other hand, if these varieties are kept at higher temperatures, *i.e.*, above the chilling temperature, consignments are liable to become over-ripe before the normal and relatively brief storage period has elapsed. It follows, therefore, that in developing avocado orchards and in organising an export industry, only those varieties should be used which are known to possess adequate cold resistance. In practice, this means that the majority of avocado types scattered throughout the West Indies, though excellent for local consumption, should not be used for export. This leaves a relatively small minority possessing the low temperature resistance and keeping qualities necessary for establishing West Indian fruit on distant markets. Thus, from current investigations, *it is recommended that some or all of six varieties should be used during the coming avocado season for trial shipments, the fruits to be picked full-grown, but quite firm, or in colouring varieties, full-grown but green, suitably packed and protected in slatted crates and transported at a steady temperature of 45°F. These stocks should also be used for propagating purposes. Casual shipments of varieties not adequately tested for keeping quality and low temperature resistance should be discontinued: not only are such consignments almost certain to arrive on distant markets in bad condition and thereby create prejudice against West Indian produce, but repeated failures will also result in the discouragement locally of a possible export industry.*

4. The phenomenon of chilling in avocado pears presents points of considerable scientific interest. It may be manifested in different ways in different varieties. A notable feature of chilling at the storage temperatures used is that complete killing of the internal tissues as a result of over-refrigeration does not take place. On the contrary, maturation processes continue but follow an abnormal biochemical trend, finally culminating in the production of a soft but darkened pulp of poor flavour. It has been shown that the onset of chilling is closely associated with ripening processes, when the fruit, which is then passing through a critical stage, is apparently more subject to modification under the impact of external factors.

5. As anticipated in earlier researches, certain varieties lend themselves to preservation by gas-storage methods. Here again, however, a considerable range in variation is apparent, some varieties being intolerant of high concentrations of CO_2 or low concentrations of O_2. In extension of this aspect of preservation, fruits were smeared with different substances to determine if ripening could be delayed without injury to the fruit. The results obtained show that the application of relatively impermeable substances definitely delays ripening. Further search for a suitable coating is, however, necessary, as those so far tested limit gaseous interchange to an extent inimical to normal ripening.

NOTES

FACILITIES FOR TRAINING AT THE TECHNOLOGICAL LABORATORY, MATUNGA, BOMBAY

As in the past the Technological Laboratory will admit this year two students for training in the elements of spinning and the routine methods of testing cotton fibre and yarn. The selected candidates will be expected to join on the 2nd December 1935 and will conform to the Laboratory regulations regarding hours of work, etc. The course normally lasts for a period of six months and a fee of Rs. 50 only will be charged for the full course.

Candidates desirous of admission should submit written applications to the Director, Technological Laboratory, Matunga, Bombay, so as to reach him not later than the 1st November 1935.

THE WOODHOUSE MEMORIAL PRIZE

The following announcement has been received from the Director of Agriculture, Bihar and Orissa :—

In memory of Mr. E. J. Woodhouse, late Economic Botanist and Principal of Sabour Agricultural College, who was killed in action in France in 1917, a biennial prize in the form of a silver medal and books of a combined value of Rs. 100 will be awarded to the writer of the best essay on a subject of botanical interest to be selected from the list noted below. The length of the essay should not exceed 4,000 words.

The competition is open to graduates of Indian Universities and to Diploma-holders and Licentiates of recognised Agricultural Colleges in India who are not more than 30 years of age on the date of submission of their essays.

Papers should be forwarded to the Director of Agriculture, Bihar and Orissa, Patna, before November 1st, 1935.

Failing papers of sufficient merit no award will be made. Essays must be type-written on one side of paper only.

1. Intergeneric hybrids and their importance to agriculture.
2. The problem of rust of wheat in India.
3. The constancy of agricultural and botanical characters of paddy and their suitability for being used in a scheme of classification.
4. Rotation of crops in relation to the eradication of weeds.

THE MAYNARD-GANGA RAM PRIZE

In 1925 the late Sir Ganga Ram, Kt., C.I.E., M.V.O., R.B., Lahore, with that generosity for which he was so well known, handed over to the Punjab Government a sum of Rs. 25,000 for the endowment of a prize of the value of Rs. 3,000 to be called the Maynard-Ganga Ram Prize and to be awarded every three years, for a discovery, or an invention, or a new practical method which will tend to increase agricultural production in the Punjab on a paying basis. The competition is open to all throughout the world. Government servants are also eligible to compete for it.

Entries for the next award were invited by the 31st December, 1933. None of the entries was considered to be of sufficient merit and it has been decided by the Managing Committee of the prize that the award should be postponed for another year and that further entries should reach the Director of Agriculture, Punjab, Lahore, on or before the 31st December, 1935.

Applications are invited for "The Maynard-Ganga Ram Prize" of the value of Rs. 3,000 which will be awarded for a discovery, or an invention, or a new practical method tending to increase agricultural production in the Punjab on a paying basis. The prize is open to all, irrespective of caste, creed or nationality, and Government servants are also eligible for it. Essays and theses are not eligible for competition. The applicants should prove that some part of their discovery, invention, etc., is the result of work done after the prize was founded in 1925. The Managing Committee reserves to itself the right of withholding or postponing the prize, if no satisfactory achievement is reported to it. All entries in competition for the next award should reach the Director of Agriculture, Punjab, Lahore, on or before the 31st December, 1935.

MEETING OF THE PERMANENT COMMITTEE OF THE INTERNATIONAL INSTITUTE OF AGRICULTURE

The Permanent Committee of the International Institute of Agriculture at its Summer meeting recently concluded, after disposing of several questions of internal interest treated other subjects of an international order.

The Committee resolved to collaborate with the Hygiene Organisation of the League of Nations, with the object of arranging an International Exhibition of Rural Housing, to be held in Rome under the auspices of the Italian Government in 1936.

It also made certain modifications in the regulations for the Agricultural Economic Committee, one of the Institute's consultative organs, in order to secure more expeditious working, and received a report on the results of the International Diplomatic Conference for the standardisation of the methods of wine analysis in international trade, results to which considerable publicity has already been given.

In addition the Permanent Committee approved the report of the last meeting of the Joint Agricultural Consultative Committee held at Geneva, 28-29 May. This body is an organ of Liaison between the Institute at Rome and the International Labour Office.

The Committee also arranged for the representation of the Institute at various International Congresses to be held during the summer and autumn of the current year, and decided to invite the 6th International Congress of Horticulture to use the assembly hall and committee rooms of the Institute for the purposes of its forthcoming meeting.

NOTICE

Copies of the undermentioned publications are available for free distribution, provided the cost of packing and postage or railway freight is met by the indentor. Application should be made to the Secretary, Imperial Council of Agricultural Research (Publication Section), Imperial Record Department Building, New Delhi :—

1. Manual of More Deadly Forms of the Cattle Diseases in India (1903). 3rd Edition.
2. Notes on the Cattle of Mysore, by A. Kristnasamiengar and Captain H. T. Pease (1912).
3. The Indigenous Breeds of Cattle in Rajputana, by Major F. S. H. Baldrey (1911).
4. The Fodder Grasses of N. India, by Duthie.
5. Some experiments in the treatment of surra in camel (1913).
6. Treatment of surra in horses by means of arsenic and its derivation.
7. Cure of surra in horses by the administration of arsenic.
8. Immunization against Charbon Symptomatique by means of a single vaccine (1909).
9. Report on the experiment carried out to test the susceptibility of cattle for several diseases and on improved method of rinderpest serum preparation (1913).
10. Flax in India, by Robert S. Finlow (1918).
11. Catalogue of Ferns in the Herbarium of the Government of India at Saharanpur (1890).
12. Agriculture in India, by James Mackenna (1915).
13. Report of Mr. C. S. Marten on the Foreign Market for Indian Timber (1927).
14. Report on the extension of cinchona cultivation in India, by Lt.-Col. Gage.
15. Catalogue of the " Plants of Kumaun ", by Sir Richard Strachey and Duthie.
16. Report of the Proceedings of the Second Entomological Meeting (1917).
17. Report of the Proceedings of the Fifth Entomological Meeting (1924).
18. Review of Agricultural Operations in India, 1919-20 to 1921-22 and 1923-24 to 1928-29.
19. Report on the progress of Agriculture in India, 1907-09, 1909-10, 1911-12 to 1918-19.
20. Memoirs of the Imperial Department of Agriculture in India.
    - (*a*) Botanical Series
    - (*b*) Bacteriological Series
    - (*c*) Chemical Series
    - (*d*) Entomological Series
    - (*e*) Veterinary Series

    } Most of the back numbers are available and can be supplied as far as possible.
21. Agricultural Journal of India
22. Journal of the Central Bureau for Animal Husbandry and Dairying in India.

    } Ditto.
23. Notes on the collection and preservation of entomological specimens : Study of life history of insects, by Stebbing (1901).

ERRATA

The Indian Journal of Agricultural Science.

Vol. V, Part IV, August 1935

Page 543, lines 10 and 11, the title of this note should read "Importation of 'Mexican Jumping Beans' into India". (This Notification of the Government of India in the Department of Education, Health and Lands, No. F. 145/35, dated the 23rd April 1935, relates to the whole of British India including Burma and not to Burma only.)

GIPD—173 IC of AR—2-11-35—800.

ORIGINAL ARTICLES

SOME OBSERVATIONS ON THE MOSAIC DISEASE OF SUGARCANE IN THE PUNJAB

BY

JAI CHAND LUTHRA, M.Sc., D.I.C., I.A.S.,

Professor of Botany,

AND

ABDUS SATTAR, B.Sc. (Ag.), Ph.D., D.I.C.,

Assistant Mycologist, Punjab Agricultural College, Lyallpur

(Received for publication on 12th July 1935)

CONTENTS

I. Introductory

The mosaic disease of sugarcane was first discovered in Java in 1890. Now it is known in almost all the sugarcane-growing countries of the world. The damage caused by this disease, which is in the form of reduction in the yield of cane and juice and deterioration in the quality of cane products has been estimated from six to fifty per cent in different countries.

In India, the disease was first noticed at Pusa (Bihar) in 1921 by Dastur [1923] and was subsequently recorded by McRae 1926 and McRae and Subramaniam [1928] in various other provinces of India. Since then, McRae [1931, 1932

has done a considerable amount of work on this disease under Bihar conditions. McRae [1926] and McRae and Subramaniam [1928] also recorded that many of the newly introduced thin Coimbatore varieties of sugarcane in the Punjab were affected by mosaic. These sugarcanes have been extensively grown in the Punjab for many years now. At the suggestion of Dr. McRae, Imperial Mycologist, Imperial Institute of Agricultural Research, Pusa, a study of the extent of occurrence of the disease and its effect on the yield of cane and juice, etc., was taken in hand in 1927 and carried on up to 1934. Co. 223, which has been largely cultivated in the province, was selected for these observations.

II. Symptoms of mosaic

The affected sugarcane plants show a general pallor of leaves. On a close examination this pallor is found to be due to irregular, light-coloured streaks or patches. These patches vary greatly in shape, but are usually elongated and run parallel to the axis of the leaf. On different varieties of sugarcane the leaf patterns caused by mosaic differ considerably. These patterns on some varieties of sugarcane have been illustrated by McRae and Subramaniam [1928].

The symptoms of the disease can be clearly detected on the first expanded leaves of young shoots, especially if these are growing vigorously. In the Punjab, it has been found that usually the first four or five leaves show the symptoms clearly. In older leaves the characteristic mosaic markings become faint, probably due to regeneration of the normal green tint of the leaf. All the canes in an affected stool may or may not show the symptoms of mosaic.

The symptoms remain prominent from June to October and can be easily observed, but afterwards, as the cane matures and winter frost sets in, the symptoms become rather obscure. The secondary symptoms of mosaic, *i.e.*, dwarfing and cracking of the stem of affected plants, which are common in other sugarcane-growing countries, have not been observed in the Punjab. From other parts of India also, these symptoms have not been reported except very rarely.

III. Incidence and amount of mosaic

The incidence and amount of mosaic has been studied on a number of sugarcane varieties in different localities of the Punjab from 1927 to 1933. The observations were recorded every year during October. The results are given in Table I. The figures given are those of the lowest and the highest percentage of infection, the latter being reached in 1933.

From a study of the figures in Table I, it is clear that the amount of infection on a given variety varies from place to place and that certain varieties are more infected than others. Thus Uba, Treru and Co. 223 were almost totally infected ; S. 48, Co. 205, Co. 309, Co. 270, Co. 262, Co. 349 and Co. 210 had more than 20 per cent infection ; Co. 213, Co. 250, Co. 287, Co. 300, Co. 313, Co. 318, Co. 346 and Katha had more than five per cent and less than twenty per cent infection and other varieties under observation showed less than five per cent or no infection at all.

TABLE I

Incidence of mosaic disease of sugarcane in the Punjab, 1927-33 (Percentage of mosaic affected stools)

Serial No.	Variety	Lyallpur	Montgomery	Multan	Rawalpindi	Gurdaspur	Jullundur	Hansi
1	Co. 205	1	0—9	0	0—2	8—44	0—T	0
2	Co. 210	0—T	..	..	7—25	6—11	..	39—51
3	Co. 213	2	..	..	2—3	T	7—10	6—7
4	Co. 223	18—65	18—100	25—33	7—40	9—51	20—100	0
5	Co. 250	..*	..	..	..	7—11	..	..
6	Co. 251	..	..	..	..	4—5	..	..
7	Co. 262	..	3—5	..	..	1—21	..	..
8	Co. 270	..	..	..	..	7—23	..	..
9	Co. 285	0—2	..	..	T	T—4	3	..
10	Co. 287	12	..	..	..	11	..	..
11	Co. 288	..	..	..	..	3	..	..
12	Co. 290	T	..	..	0	1—2	0	0
13	Co. 300	..	..	..	..	0	9	..
14	Co. 301	..	..	..	..	..	5	..
15	Co. 309	..	..	..	..	0—24	..	..
16	Co. 312	..	..	..	..	..	2	..
17	Co. 313	..	..	..	..	..	9	..
18	Co. 318	..	..	..	..	7	..	..
19	Co. 343	..	..	..	..	..	2	..
20	Co. 346	..	..	..	..	..	10	..
21	Co. 349	..	..	..	..	..	21	..
22	Co. 350	..	..	..	..	..	2	..
23	S. 48	..	..	..	..	55	..	..
24	P. O. J. 2878	..	..	..	..	..	2	..
25	Uba	..	..	..	..	100	..	..
26	Suretha	0	..	..	..	..	1	0
27	Treru	0	..	..	..	100	..	..
28	Katha	0	0	0	..	0—13	0—4	..

* Means either not grown or observations not recorded.
T=Traces.

In addition, observations were also made on many other varieties and the results showed that Co. 203, Co. 242, Co. 244, Co. 253, Co. 260, Co. 272, Co. 275, Co. 276, Co. 294, Ponda and Dhaulu showed only traces of mosaic, and Co. 214, Co. 227, Co. 231, Co. 232, Co. 233, Co. 238, Co. 243, Co. 247, Co. 252, Co. 255, Co. 257, Co. 258, Co. 261, Co. 263, Co. 265, Co. 267, Co. 268, Co. 277, Co. 281, Co. 282, Co. 283, Co. 284, Co. 286, Co. 296, Co. 314, Co. 347, B 1217, B 6308, B 6388, A 2, A 42, Lalri, Kahu and Kansar did not show any mosaic.

IV. THE PROGRESS OF MOSAIC DURING THE GROWING PERIOD OF SUGARCANE

A. Experiments at Lyallpur.—Setts from healthy and mosaic-affected canes were planted in alternate plots and there were four replications of the experiment. The trials were conducted for three years and observations were recorded from June to October every year. The results are embodied in Table II.

TABLE

Showing the development of mosaic during the growing period of sugarcane at Lyallpur

Serial No.	Kind of seed-cane planted	1929 Percentage of mosaic-affected stools in			1930 Percentage of mosaic-affected stools in						1931 Percentage of mosaic-affected stools in					
		June	August	October	Original in the seed-cane	June	July	August	Sept.	October	Original in the seed-cane	June	July	August.	Sept.	October
1	Healthy .	10·9	16·5	93·04	0	1·9	6·0	5·7	5·3	12·1	0	21·3	44·6	52·1	52·0	52·2
2	Mosaic . .	81·8	100·0	100·0	100	69·5	95·2	98·5	99·6	100·0	100	51·1	94·6	100·0	100·0	100·0
3	Healthy .	0·93	1·4	85·7	0	4·6	5·2	6·2	8·3	10·0	0	31·3	37·5	49·5	50·0	50·5
4	Mosaic .	76·4	100·0	100·0	100	71·0	92·9	97·8	99·8	100·0	100	44·5	94·4	99·6	100·0	100·0
5	Healthy .	1·8	2·0	90·0	0	2·6	3·0	6·0	6·1	12·0	0	26·9	40·9	48·3	48·1	49·0
6	Mosaic . .	90·3	100·0	100·0	100	69·3	91·5	97·1	99·4	100·0	100	55·5	93·0	99·5	99·5	99·5
7	Healthy .	2·9	3·6	86·0	0	5·9	7·0	9·2	9·4	14·2	0	15·4	36·7	39·9	41·0	42·5
8	Mosaic . .	...	...	...	100	72·0	94·6	97·1	99·8	100·0	100	59·7	95·1	99·5	99·5	100·0
9	Healthy .	...	...	...	0	2·0	6·5	6·5	7·7	13·4	0	4·4	14·6	16·6	18·0	19·8

It is clear from the figures of the table that the disease goes on increasing from June to October.

B. Experiments at Gurdaspur.—At Gurdaspur, nine varieties of sugarcane were grown on a uniform piece of land. The percentage of mosaic in the seed-canes was recorded and the experiment was carried on for three years. Observations were, as usual, recorded from June to October. The results which are given in Table III again show, as in the case of Lyallpur experiments, that the disease goes on increasing till October.

TABLE III

Showing the development of mosaic during the growing period of sugarcane at Gurdaspur

Serial No.	Name of variety	1929 Percentage of mosaic-affected stools in				1931 Percentage of mosaic-affected stools in				1932 Percentage of mosaic-affected stools in				1933 Percentage of mosaic-affected stools in			
		Original in the seed-cane	June	Aug.	Oct.	Original in the seed-cane	June	Aug.	Oct.	Original in the seed-cane	June	Aug.	Oct.	Original in the seed-cane	June	Aug.	Oct.
1	Co. 290 . .	0·3	0	0·4	0·7	*Nil*	...	0	1·0	*Nil*	0	0	0	1·0	1·0	1·4	1·7
2	Co. 285 . .	T	1·5	3·0	4·3	*Nil*	...	0	1·0	*Nil*	0	0	0	0·3	T	1·0	1·1
3	Co. 281 . .	T	0	0.8	3.3	...	Observations not recorded										
4	Co. 270 . .	19·9	10·0	14·5	23·3	*Nil*	...	5·0	11·0	*Nil*	3·4	5·2	5·8	7·0	3·4	6·3	6·7
5	Co. 262 . .	0·5	1·3	4·7	13·0	Observations not recorded								13·0	2·6	4·5	5·6
6	Co. 223 . .	12·7	58·3	71·9	75·6	*Nil*	...	47·0	51·0	*Nil*	30·2	44·4	55·7	...	21·7	33·0	35·4
7	Katha . .	*Nil*	0·8	4·1	5·7	*Nil*	...	6·0	13·0	*Nil*	1·9	3·0	3·8	Observations not recorded			
8	Co. 205 . .	Observations not recorded				*Nil*	...	14·0	33.0	*Nil*	20·6	37·6	44·4	Observations not recorded			
9	Co. 313 . .	Not grown				Not grown				*Nil*	0·4	0·8	1·1	2·4	4·6	6·5	7·4

It was also noticed in both the above experiments that the canes showed the first symptoms of mosaic about one-and-a-half months after planting.

V. Effect of mosaic on the yield of cane, juice and *gur* (raw sugar)

Experiments to find out the effect of mosaic on the yield of cane, juice and *gur* were carried out both at Lyallpur and Gurdaspur for three consecutive years from 1930 to 1933. The plan of experiments at both the places was similar and the variety under observation was Co. 223. Setts from healthy and mosaic-affected canes were planted in alternate plots and in between every two plots a strip of land 6 feet wide was sown with pigeon-pea (*arhar*) (*Cajanus indicus*) with a view to preventing as far as possible infection by contact and insects from mosaic-affected crop to the healthy one. In 1932-33, Co. 285 cane which has been found to be very resistant to mosaic, and which is taller than pigeon-pea, was grown as a barrier. The yield of stripped cane from every plot was recorded at the time of harvest. The canes from each plot were crushed separately and the juice obtained was weighed. The weight of *gur* (raw sugar) made from juice was also determined for each plot separately. The results along with the statistical analysis of the figures, by Student's method of paired differences, are given in Table IV and Table V. The data show that the differences in the yield of cane, juice and *gur* between crops grown from healthy and mosaic-affected canes are small and not significant for all the three years' trials made at Gurdaspur and at Lyallpur.

TABLE IV

Showing the effect of mosaic on the yield of cane, juice and gur *(raw sugar) of Co. 223 at Lyallpur, 1930-33*

Year	Area of plot in acres	Kind of cane planted	Percentage of mosaic-affected canes in the grown up crop	Actual weight of stripped cane	Difference between the two means	Observed value of *t*	Theoretical value of *t*	Actual weight of juice	Difference between the two means	Observed value of *t*	Theoretical value of *t*	Actual weight of *gur*	Difference between the two means	Observed value of *t*	Theoretical value of *t*	Remarks
				M. S.				M. S.				M. S. Ch.				
1930-31	1/10	Healthy .	12·1	38 5				22 31				3 36 2				
	1/10	Mosaic . .	100·0	29 31				18 16				2 37 8				
	1/10	Healthy .	10·0	28 24				18 39				3 10 6				
	1/10	Mosaic . .	100·0	25 4				16 3				2 28 12				
	1/10	Healthy .	12·0	29 0	+5·5	2·7	3·182	15 21	+2·73	2·7	3·182	2 31 12	+0·55	3·05	3·182	
	1/10	Mosaic . .	100·0	29 17				17 27				3 1 8				
	1/10	Healthy .	14·2	45 0				26 13				4 17 0				
	1/10	Mosaic .	100·0	38 11				21 4				3 27 8				
	1/10	Healthy .	13·4	43 30				23 37				4 13 12				
1931-32	1/20	Healthy .	34·0	37 33				23 27				3 14 8				
	1/20	Mosaic . .	100·0	42 0				27 4				3 24 4				
	1/20	Healthy	35.8	37 37				22 38				3 8 8				
	1/20	Mosaic . .	99·5	33 30				21 24				3 0 4				
	1/20	Healthy .	18·0	38 18	—1·81	0·84	3·182	23 14	—0·43	0·36	3·182	3 16 0	+0·40	0·30	3·182	
	1/20	Mosaic . .	100·0	34 33				20 26				3 1 8				
	1/20	Healthy .	42·0	22 30				19 22				2 39 8				
	1/20	Mosaic . .	99·0	34 18				21 31				3 7 8				
	1/20	Healthy .	24·5	39 17				23 15				3 17 8				

1932-33	1/20	Healthy .	Not recorded	25 30	—*1·60*	*1·58*	*3·182*	16 23	—*1·71*	*2·06*	*3·182*					*Gur* was not prepared.
	1/20	Mosaic .		28 15				18 31								
	1/20	Healthy .		23 30				16 4								
	1/20	Mosaic .		27 10				18 14								
	1/20	Healthy .		21 35				14 6								
	1/20	Mosaic .		22 5				14 13								
	1/20	Healthy .		20 15				13 21								
	1/20	Mosaic .		22 29				14 9								

TABLE V

Showing the effect of mosaic on the yield of cane, juice and gur *(raw sugar) of Co. 223 at Gurdaspur, 1930-33*

Year	Area of plot in acres	Kind of cane planted	Percentage of mosaic-affected canes in the grown up crop	Actual weight of stripped cane	Difference between the two means	Observed value of *t*	Theoretical value of *t*	Actual weight of juice	Difference between the two means	Observed value of *t*	Theoretical value of *t*	Actual weight of *gur*	Difference between the two means	Observed value of *t*	Theoretical value of *t*	Remarks.
				M. S.				M. S.				M. S. Ch.				
1930-31	1/20	Healthy .	7	19 28				9 15								
	1/20	Mosaic . .	91	17 19				8 35								
	1/20	Healthy .	14	24 0				12 16								
	1/20	Mosaic . .	95	23 10	+1·21	0·92	3·182	12 18	+0·33	0·41	2·782					*Gur* was not prepared as canes were affected by severe frost
	1/20	Healthy .	17	24 30				13 6								
	1/20	Mosaic . .	88	23 21				11 37								
	1/20	Healthy .	15	24 22				12 17								
	1/20	Mosaic . .	88	26 13				14 1								
1931-32	1/16	Healthy .	34	36 3				21 16				3 1 8				
	1/16	Mosaic . .	98	31 21				18 17				2 30 0				
	1/16	Healthy .	33	27 35				16 32				2 22 8				
	1/16	Mosaic . .	94	27 9	−0·67	1·05	4·303	15 38	−0·12	0·24	4·303	2 17 0	−0·013	0·24	4·303	
	1/16	Healthy .	31	24 21				15 8				2 14 0				
	1/16	Mosaic . .	95	25 26				15 18				2 16 8				
	1/16	Healthy .	30	23 31				13 20				2 9 4				
1932-33	1/16	Healthy .	40	36 37				20 30				3 8 0				
	1/16	Mosaic . .	100	35 20				20 26				3 9 12				
	1/16	Healthy .	38	38 0				23 1				3 27 0				
	1/16	Mosaic . .	100	32 11	+1·48	0·64	4·303	20 3	+1·03	0·91	4·303	3 2 8	+0·167	0·75	4·303	
	1/16	Healthy .	38	36 27				22 26				3 20 0				
	1/16	Mosaic . .	100	37 35				21 36				3 21 4				
	1/16	Healthy .	40	24 0				19 15				3 5 9				

These results lead to the conclusion that in the Punjab mosaic-affected cane crop, having as much as 100 per cent infection, does not bring about any appreciable decline in the yield of cane and other products. Likewise healthy canes with 30 per cent infection gave no significant increase over mosaic canes cent per cent infected.

VI. Effect of mosaic on the quality of juice

An analysis of the samples of juice obtained from healthy and mosaic-affected canes was also made. The samples of cane were taken at random from the plots. The results, along with the statistical analysis of the figures as calculated according to Student's method of paired differences, are given in Tables VI and VII. The data show that the differences, of the percentage of sucrose, glucose and purity coefficient, etc., between the juice obtained from healthy and mosaic-affected canes are not significant. Thus it is safe to say that in the Punjab, mosaic does not cause any significant deterioration in the quality of juice of Co. 223.

VII. Roguing as a method of control

Observations and experimental evidence referred to in the paper have shown that roguing is not quite an effective measure to control mosaic in the susceptible varieties. This measure, however, seems to keep within limit the disease in those varieties which are somewhat resistant. In the case of Co. 223, although roguing had been very carefully done in the experimental plots at the Gurdaspur Farm, yet the percentage of mosaic had remained at about fifty. In Co. 290 and Co. 285, the disease has not gone above two per cent.

TABLE

Showing the effect of mosiac on the quality

Serial No.	Kind of cane	Date of analysis	Percentage of juice	Difference between the two means (Healthy and mosaic)	Observed value of t	Theoretical value of t	Percentage of sucrose	Difference between the two means	Observed value of t	Theoretical value of t	Per cent glucose	Difference between the two means	Observed value of t	Theoretical value of t
1	Healthy . .	16th Jan. 1930.	62·2				10·08				0·167			
2	Mosaic . .	Do.	61·7				9·49				0·221			
3	Healthy . .	Do.	61·5				9·93				0·206			
4	Mosaic . .	Do.	64·2				10·21				0·283			
5	Healthy . .	Do.	63·7				9·70				0·233			
6	Mosaic . .	Do.	65·0	—0·445	1·7	2·571	9·84	+0·26	0·9	2·571	0·353	—0·035	1·1	2·571
7	Healthy . .	13th Feb. 1930.	65·7				10·88				0·143			
8	Mosaic . .	Do.	65·93				10·61				0·153			
9	Healthy . .	Do.	66·75				10·68				0·180			
10	Mosaic . .	Do.	65·65				9·08				0·133			
11	Healthy . .	Do.	64·58				10·26				0·183			
12	Mosaic . .	Do.	64·64				10·22				0·190			

TABLE

Showing the effect of mosiac on the quality

Serial No.	Kind of cane	Date of analysis	Percentage of juice	Difference between the two means (Healthy and mosaic)	Observed value of t	Theoretical value of t	Percentage of sucrose	Difference between the two means	Observed value of t	Theoretical value of t	Percentage glucose	Difference between the two means	Observed value of t	Theoretical value of t
1	Healthy . .	9th Jan. 1930.	66·5				6·0				3·7			
	Mosaic . .	Do.	66·6				6·0				3·6			
3	Healthy . .	Do.	66·9				5·0				3·6			
4	Mosaic . .	Do.	66·1				5·3				3·6			
5	Healthy . .	14th Jan. 1933.	65·5	—0·96	1·2	3·182	8·3	+0·065	0·15	3·182	0·88	0·38	1·1	3·182
6	Mosaic . .	Do.	65·0				8·1				0·93			
7	Healthy . .	13th Jan. 1934.	62·6				6·80				0·87			
8	Mosaic . .	Do.	65·7				6·79				1·03			

VI

of juice of Co. 223, Lyallpur

Percentage total sugars	Difference between the two means	Observed value of t	Theoretical value of t	Glucose ratio	Difference between the two means	Observed value of t	Theoretical value of t	Percentage of total solids	Difference between the two means	Observed value of t	Theoretical value of t	Purity coefficient	Difference between the two means	Observed value of t	Theoretical value of t
10·247				1·66				11·57				87·1			
9·711				2·33				11·14				85·2			
10·136				2·07				11·07				89·7			
10·493				2·77				11·49				88·9			
9·933				2·40				10·96				88·5			
10·193				3·59				10·99				89·6			
11·023	+0·279	1·2	2·571	1·31	—0·413	1·3	2·571	12·05	—0.01	0·07	2·571	90·30	+3·1	1·4	2·571
10·763				1·44				12·29				86·38			
10·860				1·69				12·24				87·27			
9·213				1·46				12·22				74·27			
10·443				1·79				11·73				87·45			
10·410				1·86				11·64				87·82			

VII

of juice of Co. 223, Gurdaspur

Percentage total sugars	Difference between the two means	Observed value of t	Theoretical value of t	Glucose ratio	Difference between the two means	Observed value of t	Theoretical value of	Percentage of total solids	Difference between the two means	Observed value of t	Theoretical value of t	Purity coefficient	Difference between the two means	Observed value of t	Theoretical value of t	Remarks
9·7				61·6				11·1				44·0				
9·6				60·0				10·9				55·0				
8·6				72·0				9·6				52·1				
8·9				67·9				11·1				52·4				
9·18	—0·3	1·8	3·182	10·6	—5·0	0·7	3·182	10·0	—0·69	2·0	3·182	83·0	+1·5	0·30	3·182	Samples unripe
9·03				11·5				9·9				81·8				
7·67				12·8				9·33				72·9				
7·82				15·2				9·60				70·0				

VIII. Summary

1. In the Punjab only primary symptoms of mosaic, *i.e.* mottling of leaves, occur on sugarcane. The secondary symptoms, *i.e.* dwarfing of canes, etc. have not been observed so far.

2. The incidence and amount of mosaic on different varieties of sugarcane grown in the Punjab have been recorded.

3. It has been found that the canes show the first symptoms of mosaic about one and a half months after planting, and then the amount of infection goes on increasing till October.

4. The results of field experiments carried out for three years on the sugarcane variety Co. 223 have shown that there is no significant decrease in the yield of cane, juice or *gur* (raw sugar) on account of mosaic. The quality of juice in Co. 223 also does not deteriorate on account of mosaic.

5. Roguing can keep the disease within limits in those varieties only which are not very susceptible to mosaic.

The authors wish to record their indebtedness to Dr. P. E. Lander, Agricultural Chemist, Agricultural College, Lyallpur, for analysis of various samples of sugarcane juice for us, and to Mr. D. P. Johnston and S. S. Kharak Singh for providing facilities for growing sugarcane at Lyallpur and Gurdaspur, respectively.

REFERENCES

Dastur, J. F. (1923). *Agric. J. Ind.* **18**, 505-509.

McRae, W. (1926). *Agric. J. Ind.* **21**, 198-202.

———— (1931). *Ind. J. Agric. Sci.* **1**, 527-533.

———— (1932). *Ind. J. Agric. Sci.* **2**, 378-384.

McRae, W. and Subramaniam, L. S. (1928). *Agric. J. Ind.* **23**, 239-255.

PHYSICAL PROPERTIES OF SUGARCANE MOSAIC VIRUS

BY

S. A. RAFAY,

Research Assistant, Imperial Institute of Agricultural Research, Pusa

(Received for publication on 27th April 1935)

(With one text-figure)

The resistance of a virus to physical and chemical agents varies greatly. At one end is the highly resistant tobacco mosaic virus and at the other are the viruses of tomato spotted wilt and cucumber mosaic. The present investigation was undertaken to ascertain the position of sugarcane mosaic virus. Such studies involved in every instance infection experiments with the sugarcane plants since by the results of these experiments alone can the effect of any treatment be known.

The artificial transmission of sugarcane mosaic virus has been attended with great difficulties [Matz, 1919 ; Smyth, 1920 and Lyon, 1921]. Brandes [1919] was successful to a certain extent and Bruner [1922], from his experiments, came to the conclusion, that some unknown factor influenced transmission. Fawcett [1928] could easily transmit the disease to young plants but failed with a little older ones. McRae [1926] and McRae and Subramaniam [1928] obtained cent per cent inoculation, while Sein [1930] improved on the methods previously employed. The latter admitted that the extraction of the virulent juices was a troublesome procedure and since the virus seemed to be readily destroyed or rendered non-infectious, the result would depend on the rapidity and care with which the work was done.

As the results of transmission experiments carried out by different workers have varied from 0 to 100 per cent, it is necessary to give the details of the technique employed in the present work.

It was observed that temperature, humidity and sunshine play an important role in the manifestation of the disease. It was noted during the course of another experiment that dry weather with high temperature and sunshine is favourable for the transmission of the disease provided that the plants are not older than eight to ten weeks. The value of an experiment has been frequently vitiated by an increase in humidity and fall in temperature. Where experiments have been interfered with by a sudden change in weather conditions, such experiments have been repeated. The period of successful artificial transmission of the disease lies roughly between 1st April and 1st June.

Because of these variations the procedure was standardised within practical limits. The material for studies was taken from the Co. 213 variety of sugarcane showing profuse mottling on the leaves. The plants selected were two to four months old. The first four leaves from the top were cut off at their bases and about four inches of each from the tip was cut off and discarded. These portions show no mottling and, being dry, only add to the bulk of the fibre. The leaves were then cut into small pieces with scissors and pounded in a porcelain mortar, distilled water being added gradually with renewed grinding in the quantity of one c.c. per gram of tissue. The juice was extracted through a cheese cloth, pressing with hand, and collected in a clean beaker. The juice extracted was approximately ninety per cent of the water added. All the utensils were carefully washed.

About twenty black Entomological pins No. 10 were mounted (as shown in Fig. 1) in a cork which served as a handle. With a sterile piece of cotton three to four drops of the mosaic juice (after it had received the appropriate treatment) were rubbed on the first open leaf and the spindle, the rest dropped into the hollow produced by the spindle and the open leaf. Inoculation was made by pricking with the sterile pins through juice dropped on the leaf. They were pricked ten to twelve times so that each plant received about 200 pricks. The test plants were also of Co. 213 variety. They were six to eight weeks old growing in large-sized pots.

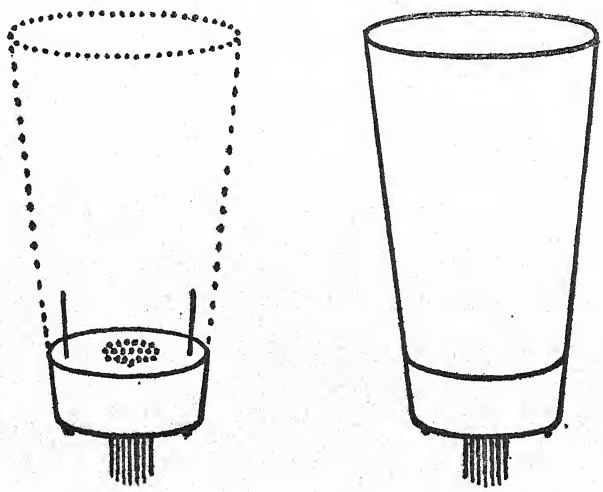


Fig 1. Showing arrangement for pricking leaves.

As purification of the virus cannot be accomplished with sufficient rapidity to enable the inoculation to be completed before the virus has lost its potency from aging, experiments were carried out with the crude virus.

Dilution

One c.c. of the juice was added to 9 c.c. of distilled water and well shaken, obtaining one-in-ten dilution. From this further dilutions were similarly made. These were inoculated to plants within thirty minutes.

Dilutions	Colour of inoculum	Date of inoculation	No. of plants inoculated	Results
1 in 10.	Green	22nd May 1934.	10	Four plants mosaic
1 in 100	Greenish	Do.	10	All healthy
1 in 1,000	Dirty	Do.	10	Do.
1 in 10,000	Colourless	Do.	10	Do.
1 in 100,000	Do.	Do.	10	Do.
1 in 1,000,000	Do.	Do.	10	Do.
Control (undiluted)	Deep green	Do.	10	Do.

Dilution of the virus extract resulted in diminution of the percentage of infection secured and by a dilution of one in hundred the virus entirely lost its potency. Crinkle mosaic of potato also tolerates one in ten dilution, while virus of tobacco mosaic is infectious at a dilution of one in 10,000.

Longevity *in vitro*

The determination of this property is fundamental for experiments on virus properties since it is important to work with the virus in extract in as virulent a form as possible.

Mosaic leaf juice was kept in an incubator at 20°C. from which inoculations were made into ten test plants at intervals shown below. The inoculations were made on 18th May 1934. The number of mosaic-infected plants in each series is given in the following table :—

10 min.	30 min.	1 hr.	2 hrs.	3 hrs.	6 hrs.	12 hrs.	24 hrs.
5	4	4	0	0	0	0	0

The virus, therefore, loses its potency in two hours while that of spotted wilt of tomato does so in six hours against tobacco-mosaic virus known to remain viable for years.

Filtration

The juice was repeatedly poured over a filter funnel till the filtrate was a clear brown liquid. The filtrate was further filtered through L_3 Chamberland filter candle. The residue on the filter paper was thoroughly washed with distilled water, filtered and the residue mixed with distilled water which was then used for inoculation experiments.

Date of inoculation	Plants inoculated with	Inoculated within	No. of plants inoculated	Results
24th May 1934.	Residue left on the filter paper	50 min. .	10	Five plants mosaic
Do. .	Filter paper filtrate.	40 ,, .	10	All healthy
Do. .	L_3 filter candle filtrate.	1½ hrs. .	10	Do.
Do. .	Control . . .	55 min. .	10	Five plants mosaic

It was found that no infection was obtained with filter paper filtrate and consequently it could not be expected from the Chamberland filtrate. It is possible that for the successful inoculation two factors are necessary, the virus itself and an accessory, of which neither alone is sufficient to produce the disease and that only one is stopped by filtration.

Reaction to chemicals

In these experiments all concentrations stated as one part of the reagent in 100 or 200, etc., of the solution refer to one gram or one c.c. of actual pure reagent in the indicated volume of the solution. A double-strength concentration of these reagents was first made with distilled water. Ten c.c. of the juice was then added to ten c.c. of this solution, bringing down the concentration to the desired strength. This method was uniformly used.

The chemicals used were Merck's pure reagents.

With stronger concentration of acids and mercuric chloride the leaf tissues were sometimes killed.

Concentrations	Period of contact	Inoculations made on	No. of plants inoculated	Results
		Hydrochloric acid (40 per cent)		
1 part in 750 .	25 minutes	5th May 1934.	10	All healthy
1 ,, 1000 .	Do. .	Do. .	10	Do.
1 ,, 1250 .	Do. .	Do. .	10	Three plants mosaic
1 ,, 1500 .	Do. .	Do. .	10	Ten plants mosaic
1 ,, 2000 .	Do. .	Do. .	10	Six plants mosaic
Control . .	Series A	Do. .	10	Six plants mosaic
		Nitric acid (65 per cent HNO_3)		
1 part in 300 .	25 minutes	5th May 1934.	10	All healthy
1 ,, 450 .	Do. .	Do. .	10	Do.
1 ,, 600 .	Do. .	Do. .	10	Do.
1 ,, 800 .	Do. .	Do. .	10	Do.
1 ,, 1000 .	Do. .	Do. .	10	Five plants mosaic
Control . .	Series A .	Do. .	10	Six plants mosaic
		Mercuric chloride		
1 part in 800 .	25 minutes	5th May 1934.	10	All healthy
1 ,, 1000 .	Do. .	Do. .	10	Do.
1 ,, 1200 .	Do. .	Do. .	10	One plant mosaic
1 ,, 1400 .	Do. .	Do. .	10	Two plants mosaic
1 ,, 1600 .	Do. .	Do. .	10	Four plants mosaic
1 ,, 2000 .	Do. .	Do. .	10	Two plants mosaic
Control . .	Series A .	Do. .	10	Six plants mosaic

Concentrations	Period of contact	Inoculations made on	No. of plants inoculated	Results
		Sodium chloride		
1 part in 25 . .	25 minutes	5th May 1934.	10	All healthy
1 „ 50 . .	Do. .	Do. .	10	Two plants mosaic
1 „ 75 . .	Do. .	Do. .	10	Eight plants mosaic
1 „ 100 . .	Do. .	Do. .	10	Six plants mosaic
Control . .	Series A .	Do. .	10	Six plants mosaic
		Copper sulphate ($CuSO_4$. 5 H_2O)		
1 part in 500 .	25 minutes	5th May 1934.	10	All healthy
1 „ 1000 .	Do. .	Do. .	10	Do.
1 „ 1500 .	Do. .	Do. .	10	Do.
1 „ 2000 .	Do. .	Do. .	10	Ten plants mosaic
Control . .	Series A .	Do. .	10	Six plants mosaic
		Hydrogen peroxide (12 volumes)		
1 part in 25 . .	20 minutes	21st May 1934.	10	All healthy
1 „ 50 . .	Do. .	Do. .	10	Four plants mosaic
Control . .	Series B .	Do. .	10	Five plants mosaic
		Formalin (40 per cent formaldehyde)		
1 part in 25 . .	20 minutes	21st May 1934.	10	All healthy
1 „ 50 . .	Do. .	Do. .	10	Do.
Control . .	Series B .	Do. .	10	Five plants mosaic

Concentrations	Period of contact	Inoculations made on	No. of plants inoculated	Results
		Zinc powder		
1 part in 20 . .	20 minutes	21st May 1934.	10	Six plants mosaic
1 ,, 50 . .	Do. .	Do. .	10	Seven plants mosaic
Control . .	Series B .	Do.	10	Five plants mosaic
		Manganese dioxide		
1 part in 20 . .	20 minutes	21st May 1934.	10	Ten plants mosaic
1 ,, 50 . .	Do. .	Do. .	10	Eight plants mosaic
Control . .	Series B .	Do. .	10	Five plants mosaic

Copper sulphate, 1 in 1,500 ; hydrochloric acid, 1 in 1,000 ; mercuric chloride, 1 in 1,000 ; nitric acid, 1 in 800 ; sodium chloride, 1 in 25 ; hydrogen peroxide, 1 in 25 and formalin, 1 in 50 ; inactivated the virus. Zinc powder and manganese dioxide, 1 in 20 did not retard the activity, and the latter may even have enhanced it. These figures compared with those given by Allard [1918] for tobacco-mosaic virus are in a very low concentration indeed and place this virus among the least resistant ones. It was interesting to note that an oxidising agent (hydrogen peroxide, Merck's) had no effect on the virus in 1 to 50 parts, while Johnson [1926] found even a resistant type like tobacco-mosaic virus to be sensitive to the inactivating influence of oxygen.

It was observed from the above results that a larger number of infections was obtained with chemicals in strengths a little more dilute than those to which the virus was just resistant*. This suggests that the virulence may be increased by dilute concentrations of such reagents.

Summary

The technique of transmission experiments with sugarcane-mosaic virus for the study of its physical properties has been standardized.

Like crinkle-mosaic of potato this virus tolerates 1 to 10 dilution. It loses its potency in two hours, thus resembling spotted wilt of tomato which is inactivated in five hours.

* (HCl, 1 in 1,500 ; $CuSO_4$, 1 in 2,000 ; NaCl, 1 in 75 etc.)

Filter paper filtrate is non-infective while the green residue left on the filter paper is infective.

This is one of the most sensitive viruses and shows the least resistance to chemical agents.

ACKNOWLEDGMENT

The writer is indebted to the Imperial Council of Agricultural Research under whose scheme for research on mosaic and other diseases of sugarcane the work has been carried out.

REFERENCES

Allard, H. A. (1918). *J. Agric. Res.* **13**, 619-637.
Brandes, E. W. (1919). *U. S. Dept. Agric. Bull.* **829**.
Bruner, S. C. (1922). *Rev. Agric. Com. & Trab. Cuba* **5**, 11-22.
Fawcett, G. L. (1928). *Rev. Ind. Agric. Tucuman* **18**, 295-299.
Johnson, James (1926). *Science*, N. S. LXIV, 1652.
Lyon, H. L. (1921). *J. Dept. Agric., Porto Rico* **3**, 65-82.
McRae, W. (1926). *Agric. J. Ind.* **21**, 198-202.
McRae, W. and Subramaniam, L. S. (1928). *Ibid.* **23**, 239-255.
Matz, J. (1919). *J. Dept. Agric., Porto Rico* **3**, No. 4, pp. 65-82.
Sein, F. (1930). *J. Dept. Agric., Porto Rico* **14**, No. 2, pp. 49-68.
Smyth, E. G. (1920). *Ann. Rep. Insular Expt. Stn., Porto Rico*, pp. 83-89.

STUDIES IN THE TECHNIQUE OF FIELD EXPERIMENTS

IV. A STUDY OF MARGIN EFFECT IN VARIETY TRIALS WITH COTTON AND WHEAT

BY

J. B. HUTCHINSON,

AND

V. G. PANSE,

Institute of Plant Industry, Indore, Central India

(Received for publication on 20th July 1935)

(With one text-figure)

CONTENTS

I. Introduction

The conclusions drawn from previous work on margin effects have recently been summarised in a paper by Christidis [1935], and will not be repeated here. He concludes that experiments should be laid out so that margins can be discarded, as they may introduce bias into the results if included. One point only not mentioned in his summary appears to require comment, and that is the attempt by some workers [Immer, 1932 ; Justesen, 1932 ; and Kalamkar, 1932] to study margin effect in data from uniformity trials. The main components of margin effect which are of interest to the agricultural worker are :—

(1) Influence of inclusion of margins on mean values.

(a) General increase due to average reduction in competition at margins, or general decrease due to trampling or other outside interference.

(b) Differential effects due to effects of competition between types differing in vigour.

(2) Influence of inclusion of margins on plot variance.

(*a*) Increase in variance due to differential effects of competition between neighbouring plots differing in vigour.

(*b*) Decrease in variance due to greater proximity of the plots to be compared.

(3) Reduction in variance due to increase in plot size when margins are included.

A study of margin effect in uniformity trials can provide information concerning items 2 (*b*) and (3) only, whereas the chief practical importance of the problem lies in determining the magnitude of the effects of competition enumerated under items 1 (*a*), 1 (*b*), and 2 (*a*). It is intended in the present paper to study the relative importance of the different effects of competition.

II. Material

Data are available from four cotton trials carried out in 1933, and one cotton trial and one wheat trial carried out in 1934-35. Of the cotton trials, two were carried out on light soil under irrigation (Ganganagar), one on black soil under irrigation, and two on black soil without irrigation. The wheat trial was also carried out on black soil without irrigation.

Of the 1933 cotton trials, 1 B/33G and 4 B/33G were variety trials conducted at Ganganagar in the Canal Colony in the north of Bikaner State. 1/33 D was a pre-rain sowing *vs.* normal sowing test of American and *desi* cotton in Dhar State in Malwa (a black soil tract) and 2/33 D was a comparison of Institute selections from Malvi cotton with the local *desi* at the same centre. The 1934 cotton trial, V31/34 I, was a trial of seven *desi* varieties carried out at Indore. Details of all are given in the Appendix.

It should be noted here that the area of the experimental plot was approximately 1/100 acre in experiments 4 B/33 G, 2/33 D, and V31/34 I, while in the other two it was much smaller. Another important difference lay in the degree of similarity of growth habits and vigour of varieties or treatments in the same experiment. In the pre-sowing trial at Dhar (Experiment 1/33D) there was a difference of three weeks between the two sowings, so that the earlier-sown plots had extra free space during the period on either side. Between the two varieties used in the experiment Malvi 9 (*desi*) was always taller and probably vegetatively more vigorous than the corresponding American (Indore 25) variety. In Experiment 1 B/33G, out of the seven varieties three were *desi*, three Punjab-Americans and one Egyptian. *Desis* grew tallest, next came the Punjab-Americans while the Egyptian remained stunted. Differences in vigour were probably similar. In experiment 4B/33G, all varieties were *desi*, behaving more alike than in the previous experiment. Mollisoni was most vigorous while Malvi was least. In experiment 2/33D, all except local were selections from the same stock of Malvi

while the local variety also was a Malvi with admixture of American cotton. Differences in growth habit and vigour were therefore expected to be least in this case. The seven varieties included in V31/34 I were all *desi* types, rather similar in vigour.

In both experiments at Ganganagar one row of the same variety on either side and two feet at each end constituted the margin round each experimental plot. In experiment 2/33 D the margin consisted of two rows on either side and three feet at each end of each plot. In experiment 1/33D four rows on either side for Malvi plots, two rows on either side for American plots and three feet at each end of all plots formed the margin. The area removed as margins was the same for all plots in this experiment also, but the spacing between rows was 28 in. in the American plots as against 14 in. in Malvi plots. Spacing between rows was uniform for all varieties in the other three experiments—2 ft. at Ganganagar and 14 in. at Dhar. All cultural operations were identical for the border rows and the experimental plot in each experiment. The distinction between margins and the experimental plot within, was made only for quantitative observations and for harvesting.

The 1933 trials were not laid out with the intention of studying margin effect. The margins of edge plots were not always protected from the stimulus of free space and are therefore not comparable with the margins of inside plots. In analysing data for plots and margins in experiments 4B/33G and 2/33D actual yields of plots having unprotected margins have been ignored owing to their abnormality and probable yields calculated by the missing plot technique [Yates, 1933]. In experiment 1/33 G, the margins of outside plots were protected by other crops of cotton. Experiment 1/33D was laid out as a Latin square, and twelve out of sixteen plots had unprotected margins but they were equally distributed between treatments and varieties, and most of the effect on variance is eliminated in the removal of sums of squares due to rows and columns. Mean increases due to inclusion of margins will, however, be somewhat exaggerated.

The 1933 results demonstrated clearly the existence of important margin effects, and therefore trial V31/34 I was harvested with a view to studying the magnitude and depth of penetration of these effects in more detail. The trial was laid out with 1/100 acre plots with two rows (14 in. apart) on each side and 4 ft. at each end of each plot as margins. Two rows of bulk cotton were sown at each side of each block to protect the margin rows of the first and last plots. Three rows on either side of each plot were harvested row by row. End margins were harvested in three strips, (1) the outer 16 in., (2) the second 16 in., and (3) the third 16 in. Corners were omitted (Fig. 1).

Finally, in order to test the applicability of the conclusions reached to another crop, yield data were collected for margin rows on a wheat variety trial in the *rabi* season of 1934-35. In this experiment eight varieties were tested in 1/100 acre plots in six randomised blocks. Margins consisted of one row on each side

and three feet at each end of each plot. Two rows of bulk were sown at each end of each block to protect the margin rows of the first and last plots. Two rows on either side of each plot were harvested and threshed separately. The effect of end margins was not considered in this experiment.

III. Results

Yields of seed cotton in each experiment were analysed in three ways; (1) Yields of experimental plots only, margins being discarded. (2) Yields of experimental plots and their surrounding margins taken together—in effect an experiment with larger plots but without margins. (3) Percentage increases in yield per unit area when yields of margin areas are added to those of the experimental plots. Percentage increases were calculated for each plot for the purpose of this analysis. It should be noted that the 'mean percentage increases' tabulated in Tables I to V for cotton and in Table XI for wheat are not proportional to the differences between acre-yields with, and without margins, since the weightage in the two cases is different.

Mean yields of seed cotton, analysis of variance, mean increases per cent, and significance of differences for each experiment are given in Tables I to V. Under "significance of differences" varieties not differing significantly in yield are included within a bracket.

Table I

Experiment 1 B/33G

Yield of seed cotton (lbs. per acre)

	Cwn. 520	Mollisoni	P 289F	P285F$_2$	0/10/26·1	P289F$_1$	New Ash.	Mean	Standard error	Significant difference
Without margins.	1800	1794	1487	1087	825	262	0	1037	75·7	225
With margins .	2187	2299	1663	1244	1182	357	0	1276	65·9	195
Percentage increase due to inclusion of margins.	21·2	29·6	11·4	15·2	46·1	97·9*	0	31·6	34·4	...

Analysis of variance of plot yields (in chitaks)

Due to	Degrees of freedom	Without margins		With margins		Percentage increase	
		Mean square	P	Mean square	P	Mean square	P
Blocks	3	37·0		105·0		7503·4	
Varieties	6	3286·0	<0·01	12499·0	<0·01	4180·3	Large
Error	18	36·7		72·4		4733·7	
Standard error per cent .	...	7·3		5·2		108·9	

Significance of differences

Without margins.—

Cwn. 520, Mollisoni, P 289F, P285F$_2$, 0/10/26·1, P289F$_1$, New Ash.

With margins.—

Mollisoni, Cwn. 520, P289F, P285F$_2$, 0/10/26·1, P289F$_1$, New Ash.

*This very large increase was almost entirely due to the failure of one plot, and the survival of its margins.

TABLE II

Experiment 4 B/33G

Yield of seed cotton (lbs. per acre)

	N. T. 10	Mollisoni	*Desi*	Roseum	Malvi	Mean	Standard error	Significant difference
Without margins .	1778	1723	1583	1478	1242	1560	49	144
With margins. .	1859	1909	1639	1555	1223	1636	44	131
Percentage increase due to inclusion of margins.	4·6	10·8	3·4	5·4	5·3	5·9	2·4	...

Analysis of variance of plot yields (in chitaks)

Due to	Without margins			With margins			Percentage increase		
	Degrees of freedom	Mean square	*P*	Degrees of freedom	Mean square	*P*	Degrees of freedom	Mean square	*P*
Blocks . .	5	92		5	131		5	24·84	
Varieties .	4	1735	<0·01	4	6068	<0·01	4	48·98	Large
Error . .	20	92		18 (20-2)	153		18 (20-2)	34·04	
Standard error per cent.	3·15			2·66			40·7		

Significant differences

Without margins .—N. T. 10, Mollisoni, *Desi*, Roseum, Malvi.

With margins.— Mollisoni, N. T. 10, *Desi*, Roseum, Malvi.

TABLE III

Experiment 1/33D

Yield of seed cotton (lbs. per acre)

	Ind. 25 pre-sown	Malvi 9 pre-sown	Ind. 25 normal	Malvi 9 normal	Mean	Standard error	Varieties			Sowings		
							Ind. 25	Malvi 9	Significant difference	Pre-rain	Normal	Significant difference
Without margins .	1271	1491	713	1141	1154	108	992	1316	265	1380	927	265
With margins. .	2048	2431	700	1329	1627	108	1374	1880	263	2239	1014	263
Percentage increase due to inclusion of margins.	61·9	60·9	7·2	16·2	36·6	13·05	34·6	38·7	...	61·4	11·9	31·8

Analysis of variance of plot yields (in tolas *for ' without margins ' and in* chitaks *for ' with margins')*

Due to	Degrees of freedom	Without margins		With margins		Percentage increase	
		Mean square	*P*	Mean square	*P*	Mean square	*P*
Rows	3	35		346		436	
Columns	3	34		711		2716	
Varieties	1	625	<0·05	1521	<0·01	69	large
Sowings	1	1225	<0·01	8930	<0·01	9816	<0·01
Varieties × sowings	1	64	large	90	large	104	large
Error	6	70		69		681	
Standard error per cent.		9·4		6·6		35·7	

Significant differences

Without margins.—Malvi 9 superior to Ind. 25 ; Pre-rain sowing greatly superior to normal sowing.

With margins.—Malvi 9 greatly superior to Ind. 25 ; Pre-rain sowing greatly superior to normal sowing.

Percentage increase.—There is a greater percentage increase in pre-rain sowing than in normal sowing.

TABLE IV

Experiment 2/33D

Yield of seed cotton (lbs. per acre)

	Malvi 8	Malvi 9	Malvi 1	Malvi 3	Malvi 5	Local	Mean	Standard error	Significant difference
Without margins .	456	454	452	446	385	352	424	28	...
With margins. .	471	466	437	433	393	353	426	25	73
Percentage increase due to inclusion of margins.	0·55	3·12	2·37	1·82	1·97	1·93	1·96	2·05	...

Analysis of variance of plot yields (in chitaks.)

Due to	Without margins			With margins			Percentage increase		
	Degrees of freedom	Mean square	P	Degrees of freedom	Mean square	P	Degrees of freedom	Mean square	P
Blocks . .	5	12·1		5	463		5	31·59	
Varieties . .	5	76	>0·05	5	263	<0·05	5	4·22	>0·05
Error . .	25	30·5		21 (25-4)	80		21 (25-4)	25·31	
Standard error per cent.		6·6			5·9			104·7	

Significant differences

Without margins.— Malvi 8, Malvi 9, Malvi 1, Malvi 3, Malvi 5, Local.

With margins.—Malvi 8, Malvi 9, Malvi 1, Malvi 3, Malvi 5, Local.

TABLE V

Experiment V31/34I

Yield of seed cotton (lbs. per acre)

		G16	G 51	Malvi 9	Malvi 1	Cwn. 520	Local	Roseum	Mean	Standard error	Significant difference
Without margins		520	505	478	477	441	387	384	456	23·9	69
With margins.	Sides .	524	515	481	493	453	384	394	464	21·1	61
	Ends .	513	497	477	470	435	383	373	450	22·3	64
Percentage increase due to inclusion of margins.	Sides .	0·3	2·0	0·3	3·1	2·4	—1·1	1·7	1·2	1·5	...
	Ends .	—1·9	—1·6	+0·1	—1·9	—1·2	—1·3	—3·1	—1·6	1·3	...

Analysis of variance of plot yields (in 10 grams)

Due to	Degrees of freedom	Without margins		With side margins		With end margins		Percentage increase side margins		Percentage increase end margins	
		Mean square	P	Mean square	P	Mean square	P	Mean square	P	Mean square	P
Blocks . . .	5	1368		4142		1237		23·6		35·8	
Varieties . .	6	3610	< 0·01	8101	< 0·01	5769	< 0·01	12·8	large	5·6	large
Error . . .	30	702		967		959		12·8		9·7	
Standard error per cent.		5·2		4·5		4·9		118·4		81·3	

Significance of differences

Without margins.—G16, G51, Malvi 9, Malvi 1, Cwn. 520, Local, Roseum.

With side margins.—G16, G51, Malvi 1, Malvi 9, Cwn. 520, Roseum, Local

With end margins.—G16, G51, Malvi 9, Malvi 1, Cwn. 520, Local, Roseum.

That margin rows usually yield more per unit area than inside rows is well known [McClelland, 1929; 1934; Hollowell and Heusinkveld, 1933; Deming and Brewbaker, 1934; Coombs, 1934]. This is borne out in the experiments examined, every variety or treatment in all experiments except V31/34I registering an increase in acre-yield when margins are added to the experimental plots. In experiment V31/34 I also, the inclusion of side margins caused a small increase in acre-yield in all varieties save one. The inclusion of end margins, however, caused a small decrease in yield in all varieties save one. This influence of margins in causing an increase in acre-yield has persisted even after allowance has been made by the missing plot technique for the disproportionately large increases in yield of margins of unprotected outside plots.

In Table VI are given the percentage increases in yield per unit area resulting from the inclusion of margins of unprotected outside plots, and of margins of inside plots of the same varieties.

TABLE VI

Percentage increase in yield per unit area when margins are included

Experiment	Variety	Unprotected plots	Protected plots
4B/33G	Malvi	18·1	4·7
2/33D	Malvi 1	37·8	1·9
	Malvi 3	22·9	1·4
	Malvi 8	27·5	1·2
	Local	31·5	1·6

An increase in yield per unit area of from 18·1 to 37·8 per cent has resulted from the inclusion of unprotected outside margins, whereas only 1·2 to 4·7 per cent increase resulted from the inclusion of margins of inside plots. Clearly the increased yield of margins of protected plots demonstrated in Tables I to V is very small compared with that of margins of plots without a cotton crop on one side, and though, as will be shown later, it may in certain cases be advantageous to dispense with plot margins, this can only be done when outside plots are adequately protected by guard rows.

The observation, that in all cases margin rows gave higher yields than inner rows of the same plots, shows that competition must be less between plants of different varieties than between plants of the same variety. A comparison of data from experiments including widely different types or treatments with that from

experiments including types of similar habit is given in Table VII and shows that where differences were greater, competition was less and increases in marginal yields greater.

TABLE VII

Percentage increase in yield per acre due to inclusion of margins

Experiment	Type of comparison	Mean increase	Range of increase
1B/33G	*Desi vs.* American varieties .	20·6	0 to + 46·1*
1/33D	*Desi vs.* American, and pre-sowing *vs.* rain-sowing.	36·6	+ 7·2 to + 61·9
4B/33G	*Desi* varieties . . .	5·9	+ 3·4 to + 10·8
2/33D	Malvi (*desi*) selections .	2·0	+ 0·5 to + 3·1
1/34I	*Desi* varieties — side margins .	1·2	— 1·1 to + 3·1
	Desi varieties — end margins .	—1·6	— 3·1 to + 0·1

*P289F_1 gave an increase of 97·9 per cent, but this was almost entirely due to the failure of a single plot, and has, therefore, been omitted from the calculation of the mean in this Table.

In the two experiments in which both *desi* and American types were included, the mean increase in acre-yield resulting from inclusion of margins was 20 and 35 per cent and varied for individual varieties from 0 to 60 per cent. Where only *desi* varieties were included in an experiment, the mean increase due to inclusion of margins was not more than six per cent, and varied in individual varieties from —1 to + 11 per cent.

Examination of the increases in different varieties (Tables I-V) shows that in general the more vigorous varieties gave the greater increases on inclusion of margins. Examples are 0/10/26·1 (vegetatively very vigorous, though low-yielding) and Mollisoni in 1 B/33G, Mollisoni in 4 B/33G, and pre-rain sown plots in 1/33D. The standard error of increases was, however, very large in all cases, and none of the differences in increase except that due to sowing date in 1/33D were significant.

The influence of inclusion of margins upon the standard error of an experiment is the resultant of the effects of two opposing forces ; (1) the greater variability of margins due to differential effects of different neighbours on the plots of each variety, and (2) the reduction in variability associated with increased plot size [Hutchinson and Panse, 1935]. The resultant effect on the standard errors of the experiments under discussion is shown in Table VIII, where plot sizes and their standard errors per cent are given for plots alone, and plots *plus* margins.

TABLE VIII

Plot sizes and standard errors per cent for plots, and plots plus margins

Experiment	Margins excluded		Margins included		
	Plot size (acre)	Standard error per cent	Plot size (acre)	Percentage increase in plot size	Standard error per cent
1/33D	0·001	9·4	0·005	400·0	6·6
1B/33G	0·005	7·3	0·008	48·1	5·2
4B/33G	0·01	3·2	0·015	45·6	2·7
2/33D	0·01	6·6	0·018	83·0	5·9
V31/34I	0·01	5·2	0·013	33·3	4·5 (including side margins)
			0·012	25·8	4·9 (including end margins)

The net effect in all cases is a considerable reduction in standard error per cent. As would be expected, the reduction is greater in experiments 1/33D and 1B/33G, as the advantage of increasing the size of the plot is greater when the plots are small.

In the calculation of data for Table VIII, the missing plot technique was used to adjust the yields of plots in experiments 4B/33G and 2/33D which had unprotected margins. A comparison of standard errors per cent of plots *plus* margins using actual and adjusted yields gave the following figures :—

Experiment	Standard error per cent of yield	
	Actual	Adjusted
4B/33G	3·4	2·7
2/33D	8·4	5·9

These figures emphasise the conclusion drawn from the corresponding figures for mean values that though it may sometimes be advantageous to provide no margins, this can never be done unless adequate guard rows are provided to protect outside plots.

In Tables I to V are given the order of yield of varieties and treatments, and the significance of yield differences, for plots excluding margins and plots *plus* margins separately. Only four changes in order occurred when margins were included, and all changes were due to small differences between varieties not differing significantly in yield. There were, however, a number of changes in significance where the order remained the same. In experiment 1B/33G, $P285F_2$ gave a significantly higher yield than 0/10/26·1 when plots were considered without margins. Owing to the high relative yield of margins of 0/10/26·1, when plots *plus* margins were considered, it was not significantly inferior to $P285F_2$. In experiment 4B/33G, the difference between Mollisoni and *desi* fell just short of significance when plots alone were considered, but was double the value required for significance when plots *plus* margins were considered. In experiment 1/33D the significance of both varietal and sowing date differences was increased by the inclusion of margins, but in this case the greater differentiation between sowing dates is misleading, since margins of early-sown plots had a significantly greater advantage than those of normal-sown plots. In experiment 2/33D, when margins were excluded, the differences between varieties could not be regarded as significant. The inclusion of margins, however, caused a slight increase in acre-yield and a decrease in standard error, so that the differences in yield between the first three varieties and the last are now distinctly significant. In experiment V31/34I the inclusion of side margins gave a slightly better differentiation between varieties. The inclusion of end margins did not alter the significance of differences.

On the whole, therefore, the inclusion of margins has increased the efficiency of the experiments in practically all cases by reducing the standard error per cent. This increase in efficiency has been gained at the expense of a slight loss in the accuracy of the mean values, but only in the case of the pre-sowing experiment (1/33D) has this loss of accuracy acted differentially between treatments and given a misleading result.

In the cotton trial V31/34I side margins and end margins were studied in detail. In Fig. 1 is given a diagram of a single plot to show the method of harvesting.

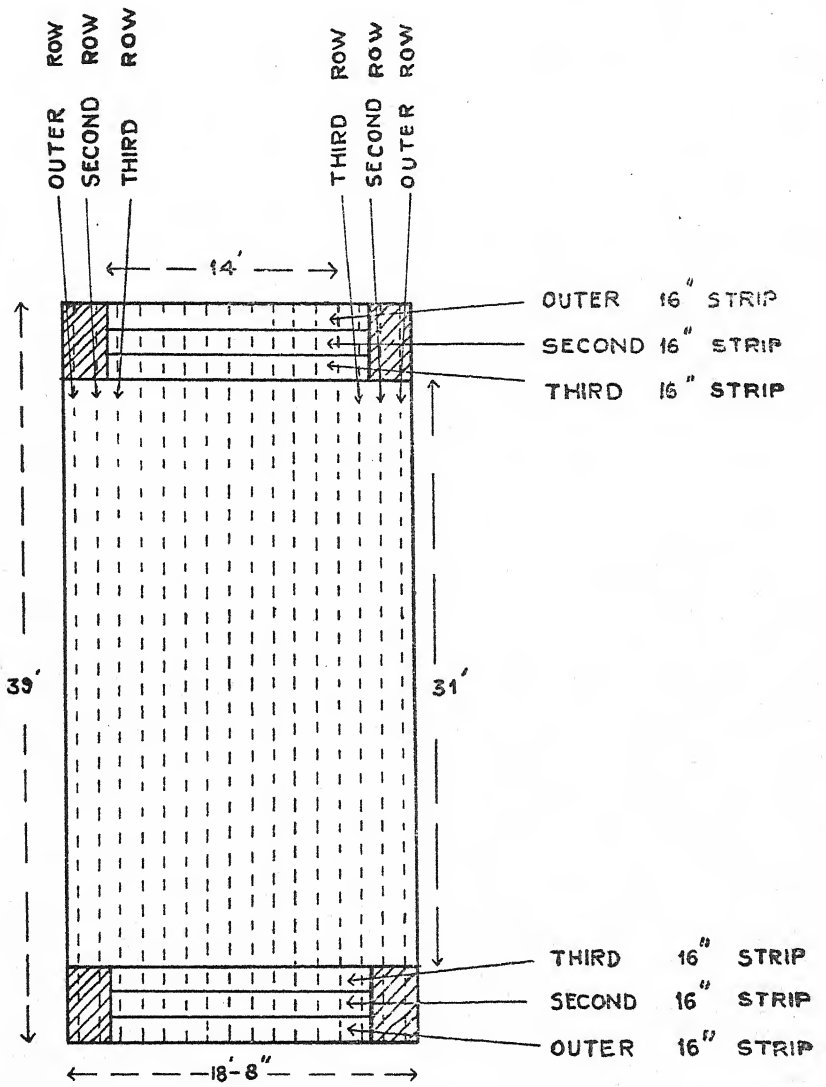


Fig. 1. A single plot in experiment V31/34Ishowing arrangement for harvesting marginal areas..

For the study of the effect of side margins, the produce of the outer rows, the second rows, and the third rows on either side of each plot was harvested separately, making three sets of data from progressively increasing distances from the edge of the plot. Similarly for the study of end-of-row margins, three strips 16 in. wide were marked off at each end of each plot, and the produce of the outer, second and third strips harvested separately to provide three sets of data from different distances from the ends of the plots. Means and standard errors per cent are given in Tables IX and X.

TABLE IX

Mean yields and variances of plots and side margins in cotton trial V31/34I

	Mean yield (lbs. per acre)	Standard error per cent
Plot with margins excluded	456	5·2
Plot with side margins included . . .	464	4·5
3rd and 14th rows	449	7·7
2nd and 15th rows	452	8·1
Outer rows	509	9·9

TABLE X

Mean yields and variances of plots and end margins in cotton trial V31/34I

	Mean yield (lbs. per acre)	Standard error per cent
Plot with margins excluded	456	5·2
Plot with end margins included . . .	450	4·9
Inner 16 in. strips	409	8·6
Middle 16 in. strips	430	10·3
Outer 16 in. strips	396	9·2

Considering side margins, both sets of inner rows gave approximately the same yield per unit area as the central plot, while the outer rows gave slightly more. The standard error per cent was very similar in the two inner sets, and was considerably and significantly greater in the outer rows. Considering end margins, the yield per unit area was rather less in all margin strips than in the central plot and did not differ much from strip to strip. Standard error per cent was much the same in all three margin strips. The whole of the margin effect, therefore, appears to be located in the outermost side rows. There appears to have been no margin effect in end of rows in spite of the fact that they were not protected by guard-rows.

In Tables XI and XII are given data for the wheat trial 2V12/34I comparable to that given for cotton trials in previous tables.

TABLE XI

Experiment 2V12/34I (wheat). Yield of grain (in lbs. per acre)

	P. 8A	Bansi 224	P. 9D	Local	Bansi Pili 808	C. P. 115	Pusa 114	Cross 591	Mean	Standard error	Significant difference
Without margins . . .	766	751	749	689	670	660	638	630	697	29·1	82
With margins . . .	758	774	746	708	673	685	635	623	700	27·2	77
Per cent increase due to inclusion of margins	0·2	4·6	0·8	4·0	1·7	1·9	0·6	0·1	1·7	1·0	2·9

Analysis of variance of plot-yields

Due to	Degrees of freedom	Without margins		With margins		Per cent increase	
		Mean square	*P*	Mean square	*P*	Mean square	*P*
Blocks	5	1443		1925		13·5	
Varieties	7	415	<0·01	655	<0·01	17·8	<0·05
Error	35	126		153		6·4	
Standard error per cent .		4·2		3·9		60·1	

Significance of differences

Without margins.— P. 8A, Bansi 224, P. 9D, Local / Bansi Pili 808, C. P. 115, Pusa 114, Cross 591.

With margins.— Bansi 224, P. 8A, P. 9D, Local, / C. P. 115, Bansi Pili 808, Pusa 114, Cross 591.

TABLE XII

Mean yields and variances of plots and side margins in wheat trial 2V12/34I

	Mean yield (lbs. per acre)	Standard error per cent
Plots with margins excluded	697	4·2
Plots with margins included	700	3·9
2nd and 13th rows	693	4·8
Outer rows	756	5·9

The margin effects observed are in all respects similar to those observed in cotton trials. Slight increases in mean yields were observed in all varieties when margins were included. These increases were greatest in Bansi 224, which was considerably earlier than any other variety in the experiment except Bansi Pili 808, and had a considerable advantage in height over its neighbours from the early stages until ripening, and in local which was tall and tillered vigorously. These two gave a significantly greater increase on inclusion of margins than any other variety except C. P. 115 and Bansi Pili 808. The Punjab varieties (8A, 9D and Cross 591) were considerably slower in development in the early stages than the other varieties, and this appears to be responsible for the very small increases in their margin rows.

The standard error per cent is slightly reduced by the inclusion of margins, but the advantage is less than in most of the cotton trials. This is partly accounted for by the fact that the inclusion of margins only increased the plot size by 17 per cent.

The inclusion of margins caused two changes in the order of yield, P. 8A and Bansi 224, and Bansi Pili 808 and C. P. 115 changing places. These changes, however, hardly affected the significance of differences. The reduction in standard error when margins are included has somewhat improved the differentiation between varieties.

In Table XII are given mean yields per acre and standard error per cent for outer rows of each plot, and the second and thirteenth rows of each plot. As in the cotton trial, only the outermost rows exhibited margin effect in increased mean yield and increased standard error per cent, the difference in standard error per cent approaching the customary $P = 0{\cdot}05$ point for significance.

IV. DISCUSSION

It has been shown that the importance of margin effect depends primarily upon the magnitude of the differences between the varieties or treatments included in the experiment. Where differences are large, large disturbances in mean values

may be expected if adequate margins are not provided, and misleading conclusions may be drawn. Where differences are small, disturbances in mean values are not likely to be important, and the efficiency of the experiment will probably be improved by using the area otherwise discarded as margins to increase the plot size. If experiments can be laid out without margins, the management of out-station experiments where skilled supervision is not available will be greatly simplified.

Where variety differences are large, and the provision of margins is essential, one row on either side of each plot should be adequate. Where end margins were studied separately no evidence of margin effect was obtained and margins actually yielded slightly less than central plots. This was probably due to irregularities in seed rate at the ends of plots and to extra trampling at the time of sowing. A safe precaution would be to leave three to four feet at each end of each plot to allow for possible mixing of seed or irregularity in sowing. Only one experiment (1/33D) was studied in which an agronomic treatment was included, and in this case large plot margins were provided, and the depth of penetration of margin effect was not studied. It is probable, however, that with the large differences between treatments often employed in agronomic trials, larger margins may be necessary than in variety trials.

Margin effect varies considerably not only from variety to variety, but also from plot to plot of the same variety in the same experiment. This is due to the fact that different plots of a variety have different neighbours, and the effects of competition on plot margins depend upon the relative vigour of the variety and its neighbour. Where a markedly inferior variety is in competition with a superior variety, the normal increase in yield of margins over central plots may be reversed in plots of the inferior variety. Almost always in the experiments under discussion, however, margins of all varieties have yielded more than their central plots, and this indicates that competition between varieties is not usually so severe as competition between plants of the same variety.

The range of conditions under which the cotton trials were carried out, and the confirmation of the results obtained from them by the data from the wheat trial justify the belief that the relative importance of different components of margin effect are very similar under most conditions likely to be met with in practice.

V. Summary

Studies are reported on the extent and importance of margin effect in variety trials on cotton and wheat.

The cotton trials were grown under a range of different conditions including irrigated and dry, and black cotton soil and sandy soil.

It is shown that the magnitude of the effect of margins on means depends primarily upon the extent of the differences between the varieties included in the trial.

Where differences between varieties were great, margin effect was large, and failure to allow adequate plot margins would have resulted in the drawing of erroneous conclusions. Where differences between varieties were small, margin effect was small, and the inclusion in the plots of areas, otherwise to be discarded as margins, caused a reduction in error which materially improved the efficiency of the experiment.

It is concluded that where markedly different varieties are to be compared, one row on either side of each plot will be adequate for side margins. For end margins three to four feet should be provided at each end of each plot to allow for the effects of trampling, irregularities in sowing and possible mixing of seed. Where varieties to be tested are similar in habit, it is actually advantageous to dispense with margins especially where other considerations make it desirable,—*e.g.*, where difficulties of control in out-stations make simplicity essential—but in such cases it is most important to surround the experiment with guard rows of bulk crop to protect the outside plots. With the wide differences in treatment common in agronomic experiments, wider margins are probably necessary.

The data show that in almost all varieties studied, competition between varieties was less than between plants of the same variety. Where margins are dispensed with, therefore, yields will be somewhat over-estimated.

VI. Acknowledgments

The yield data from the Dhar and Ganganagar experiments were collected and supplied to us by the Superintendent of Agriculture and State Gardens, Dhar State, and the Agricultural Officer, Bikaner State, respectively, and we take this opportunity of offering them our thanks for their co-operation.

REFERENCES

Christidis, B. G. (1935). *J. Agric. Sci.* **25**, 231.
Coombs, A. V. (1934). *Emp. J. Expt. Agric.* **2**, 315.
Deming, G. W., and Brewbaker, H. E. (1934). *J. Amer. Soc. Agron.* **26**, 615.
Hollowell, E. A., and Heusinkveld, D. (1933). *J. Amer. Soc. Agron.* **25**, 779.
Hutchinson, J. B., and Panse, V. G. (1935). *Ind. J. Agric. Sci.* **5**, 523.
Immer, F. R. (1932). *J. Agric. Res.* **44**, 649.
Justesen, S. H. (1932). *J. Agric. Sci.* **22**, 366.
Kalamkar, R. J. (1932). *J. Agric. Sci.* **22**, 373.
McClelland, C. K. (1929). *J. Amer. Soc. Agron.* **21**, 524.
——————— (1934). *J. Amer. Soc. Agron.* **26**, 491.
Yates, F. (1933). *Emp. J. Expt. Agric.* **1**, 129.

Appendix

Experiment 1B/33G, cotton varieties, irrigated

The experiment was surrounded by bulk cotton on three sides and another cotton varietal trial on the fourth.

Area of each plot :

(1) Margins included, 18 ft. × 19 ft. 6 in. = 0·00806 acre.

(2) Margins excluded, 14 ft. × 15 ft. 6 in. = 0·00498 acre.

Plots with margins excluded are 61·8 per cent of those with margins included.

Varieties : Mollisoni
Cwn. 520
0/10/26·1
P. 289 F
P. 289 F_1
P. 285 F_2
New Ashmouni

Layout—Four randomised blocks in two rows.

Experiment 4B/33G, cotton varieties, irrigated

The experiment had another cotton varietal trial and bulk cotton on one side and only one row of *desi* cotton with vacant space beyond on the other. A path two feet wide ran along the ends of experimental rows.

Area of each plot :

(1) Margins included, 18 ft. × 35 ft. = 0·0145 acre.

(2) Margins excluded, 14 ft. × 31 ft. = 0·01 acre.

Plots with margins excluded are 69·0 per cent of those with margins included.

Varieties : Roseum
N. T. 10
Mollisoni
Desi
Malvi

Layout—Six randomised blocks in two rows.

NOTE.—Since the end plots, both of Malvi, in blocks 1 and 4 were not well protected by extra rows beyond and the yield figures for these plots with their margins included were high, their yields estimated by the missing plot technique have been used in the statistical analysis.

Experiment 1/33D, pre-sown and rain-sown American and Desi cotton, irrigated

The experiment was not surrounded by any crop.

Area of each plot :

(1) Margins included, 14 ft. × 15 ft. = 0·00482 acre.

(2) Margins excluded, 4 ft. 8 in. × 9 ft. = 0·000964 acre.

Plots with margins excluded are 20·0 per cent of those with margins included.

Varieties and treatments :	Cambodia, Indore 25, pre-sown.
	Malvi 9, pre-sown.
	Cambodia, Indore 25, rain-sown.
	Malvi 9, rain-sown.
Dates of sowing—	Pre-rain on 29th May 1933.
	On rains on 22nd June 1933.
Layout—	Latin square 4 × 4.

Experiment 2/33D, cotton varieties, dry

The experiment was surrounded by a road on three sides and there was bulk cotton at some distance from the experiment on the fourth.

Area of each plot :

(1) Margins included, 11 ft. 8 in. × 68 ft. 3 in. = 0·0183 acre.

(2) Margins excluded, 7 ft. × 62 ft. 3 in. = 0·01 acre.

Plots with margins excluded are 54·6 per cent of those with margins included.

Varieties : Malvi 1
Malvi 3
Malvi 5
Malvi 8
Malvi 9
Local

Layout—Six randomised blocks in two rows.

NOTE.—Two end plots of Malvi 1 and Malvi 3 in blocks 3 and 6 were on the road side and two more plots of Malvi 8 and local at the other end had also extra vacant space beyond. The yield of all these four plots with margins included was abnormally high, so that their yields estimated by the missing plot technique have been used in the statistical analysis.

Experiment V31/34I, cotton varieties, dry

The experiment had two guard rows on outer side of the edge plots.
Area of each plot :

(1) Side margins included, 18 ft. 8 in. × 31 ft. = 0·0133 acre.
(2) End margins included, 14 ft. × 39 ft. = 0·0126 acre.
(3) Margins excluded, 14 ft. × 31 ft. = 0·01 acre.

Plots with margins excluded are 75·2 per cent of those with side margins included and 79·4 per cent of those with end margins included.

Varieties : Cwn 520
Malvi 9
Roseum
Local
G. 16
Malvi 1
G. 51

Layout—Six randomised blocks in three rows.

Experiment 2V12/34I, wheat varieties, dry

The experiment had two guard rows on the outer side of the edge plots.
Area of each plot :

(1) Both side and end margins included, 16 ft. 4 in. × 37 ft. =0·0139 acre.
(2) Side margins included, 16 ft. 4 in.× 31 ft. = 0·0117 acre.
(3) Margins excluded, 14 ft. × 31 ft. = 0·01 acre.

Plots with margins excluded are 85·5 per cent of those with side margins included.

Varieties : Punjab 8A
Punjab 9D
Cross 591
Pusa 114
C. P. 115
Bansi 224
Bansi Pili 808
Local

Layout—Six randomised blocks in three rows.

HYBRID VIGOUR IN WHEAT*

BY

F. L. ENGLEDOW, M.A.,

AND

B. P. PAL, M.Sc., PH.D., F.L.S.,

A SUMMARY

BY

B. P. PAL

(Received for publication on 26th August 1935)

I. INTRODUCTION

A proper understanding of hybrid vigour or heterosis is of importance both from the academic and the practical viewpoint: from the former because herein lies one of the profoundest mysteries in genetics, from the latter because breeding policy must be regulated according to whether or not vigour of the outstanding order sometimes found in the F_1 can be "captured" as a homozygous segregate. Though the scientific interest of hybrid vigour is of long standing, the extensive literature on the subject is characterised by some unsatisfactory features, the most fundamental being inadequacy of the numerical evidence. Further, it is often impossible to tell exactly what is meant by "selfing": the pollen applied to a selfed plant may have been obtained from the plant itself or from another plant of the same "selfed" line.

Within recent years hybrid vigour has been exploited with a certain measure of success by plant-breeders. In some crops notably in maize, tobacco and tomato, crossed seed can be obtained with ease in such quantity as to make it practicable to grow a commercial crop of F_1 plants. Substantial yield increases by this means have been claimed.

---

*This paper is an abbreviated account of 'Investigations on yield in the cereals. VIII. Hybrid vigour in wheat' by F. L. Engledow and B. P. Pal, published in *J. Agric. Sci.* 1934: **24**: 390-409. The writer is obliged to Prof. Engledow and to the Editors of the *Journal of Agricultural Science* for permitting the publication of the article in this form.

Although it would be impracticable to grow commercial crops of F_1 plants of a self-pollinating plant, such as wheat, it might be possible theoretically, assuming hybrid vigour is shown by this plant, to increase yields by regularly raising crops of, it may be, F_5 or F_6 plants. Upon consideration of all the circumstances it was decided to look for and study hybrid vigour in wheat. Comparisons of F_1 plants with the parents are limited in range by the difficulty of obtaining sufficient seed by crossing. It was therefore decided to work along two separate lines: first, to make an intensive study of one cross; concurrently to explore possibilities by comparing small F_0 and F_1 populations for a large range of crosses. In all cases development was studied by the easily determined but valuable index, rate of tiller formation, since an increase in 'vigour' as judged by final yield could not be interpreted without growth data.

II. Experimental work

(a) *Statistical comparison of F_1, F_2 and F_3 with the parent forms*

Little Joss (alternatively called Joss), a red smooth-chaffed variety, and Thule, white rough-chaffed variety, of *Triticum vulgare*, were reciprocally crossed. The growings were:

Generation	*Year*	*Plot layout*
The reciprocal F_1's and both parents.	1929-30	A 4 × 4 Latin square with 108 seeds per plot.
F_2 and both parents (the F_2 was an equal mixture of the seeds set by the reciprocal F_1's).	1930-31	A 4 × 4 Latin square with 560 seeds per plot. The Latin square was sown in duplicate.
F_1's and both parents . . .	1931-32	In each case a 4 × 4 Latin square with 162 seeds per plot.
F_2's and both parents		
F_3's and both parents.		

In the plots the sowing interval was 8 × 2 in.

(b) *Small-scale observational comparisons of the F_1 and the parents for a large range of crosses*

The F_1 population was of about twenty-five plants only, so that although actual measures of germination, tillering and yield were made, the data cannot be examined statistically. It was hoped that these experiments might reveal some

cases of marked hybrid vigour and show the relative value of parental forms in relation to the phenomenon. The crosses were in four groups, *viz* :—

Between varieties of *T. vulgare* (21 chromosomes)*.
Between varieties of species with 14 chromosomes.
Between varieties of species with 21 chromosomes.
Between the 14 and 21 chromosomes species.

In almost all cases reciprocal crosses were made.

(c) *Physiological experiments*

Comparisons of F_1 and parents were made in respect of embryo weight, rate of dry weight increase, and rate of respiration of germinating grains. The respiration experiments were very kindly carried out by Dr. H. R. Barnell.

The effect upon grain development and germination of covering the ear with a waterproof paper bag during hybridising was studied, as also the alleged effect upon vigour of the act of hybridising ("regeneration ") when plants of the same variety are used as parents.

III. Detailed study of the cross Little Joss × Thule†

As explained in section II, reciprocal crosses were made between these two varieties and the F_1, F_2 and F_3 generations were compared with the parents. In 1931-32 there were daily counts of germination and of rate of leaf formation. In this and in the other two crop-years also, some observations on tillering and determinations of final yield of grain were carried out.

Germination.—The initial differences between the parents themselves and between the two (reciprocal) F_1's were not significant but at every germination count the F_1 values were significantly greater than those for either parent, in most cases at the 100 : 1 point and in all cases at the 20 : 1 point at least.

Leaf formation.—Counts made in 1931-32 showed the F_1 populations to be in advance of both parents in rate of leaf formation (prior to the commencement of tillering). The F_2 from Joss × Thule had significantly more leaves than either parent, but the reciprocal F_2 exceeded only the Thule parent. Both the F_3 generations were significantly greater than Thule but not significantly greater than Joss.

Tillering.—Tillering, taken to mean number of side shoots (*i.e.*, additional to the main axis of the plant), gave results which, for all the counts made, are shown fully in Table I. The essential differences are set out below where T = Thule, J=Little Joss, J×T=Fx Joss × Thule, T×J = F_x Thule × Joss, $>$ = significantly greater at 100 : 1 point and ($>$) = significantly greater between the 20 : 1 and 100 : 1 points. While many actual differences in Table I are not statistically

* The haploid number of chromosomes is referred to.

† In naming the parents of a cross, the female is always written first in this paper.

significant the constancy of the sign in some cases, for instance, the difference between the reciprocal F_1's in 1929-30, deserves to be noticed.

F_1 J × T	> T	at	6	out of 6	counts in 1929-30.
	> T	,,	1	,,	1 count in 1931-32.
	> J	,,	2	,,	6 counts in 1929-30.
	> J	,,	1	,,	1 count in 1931-32.
	> F_1 T×J	,,	2	,,	6 counts in 1929-30.
	> F_1 T×J	,,	1	,,	1 count in 1931-32.
F_1 T × J	> T	at	6	out of	6 counts in 1929-30.
	> T	,,	1	,,	1 count in 1931-32.
	< J	,,	2	,,	6 counts in 1929-30.
	> J	,,	1	,,	1 count in 1931-32.
	< F_1 J×T	,,	2	,,	6 counts in 1929-30.
	< F_1 J×T	,,	1	,,	1 count in 1931-32.
F_2 J × T	> T	at	4	out of	4 counts in 1930-31.
	> T	,,	1	,,	1 count in 1931-32.
	< J	,,	4	,,	4 counts in 1930-31.
	< J	,,	0	,,	1 count in 1931-32.
	> F_2 T×J	,,	1	,,	4 counts in 1930-31.
	(>) F_2 T×J	,,	1	,,	1 count in 1931-32.
F_2 T×J	> T	at	4	out of	4 counts in 1930-31.
	> T	,,	1	,,	1 count in 1931-32.
	< J	,,	4	,,	4 counts in 1930-31.
	< J	,,	1	,,	1 count in 1931-32.
	(<) F_2 J×T	,,	1	,,	4 counts in 1930-31.
	(<) F_2 J×T	,,	1	,,	1 count in 1931-32.
F_3 J×T	> T	at	1	out of	1 count in 1931-32.
	< J	,,	1	,,	1 ,, 1931-32.
	> F_3 T×J	,,	0	,,	1 ,, 1931-32.
F_3 T×J	> T	at	1	out of	1 count in 1931-32.
	< J	,,	1	,,	1 ,, 1931-32.
	< F_3 J×T	,,	0	,,	1 ,, 1931-32.

For the parents J > T at all the thirteen counts in three years combined, and significantly so in eleven of them.

TABLE I

Average number of side tillers per plant (i.e., *not counting the main axis*) *in Joss, Thule, and their* F_1, F_2 *and* F_3 *generations. The numbers are expressed on the basis of the general mean* (*of the two parents and the two hybrid generations*) = *100.*

Date of count	Joss	Thule	Joss × Thule	Thule × Joss
		F_1 1929-30		
25th January . . .	113·4	56·7	119·8	111·5
1st February . . .	111·1	66·8	116·6	107·5
15th February . . .	114·1	64·2	116·5	105·0
1st March	109·9	63·2	120·2	106·5
15th March	109·5	70·9	114·3	105·3
8th August (harvest) . .	109·1	93·9	101·0	97·0
		F_1 1931-32		
23rd February . . .	92·5	64·3	123·4	119·7
		F_2 1930-31		
27th January . . .	148·4	34·4	123·0	95·1
17th February . . .	120·7	77·5	102·4	100·0
18th March	135·4	67·0	99·7	98·3
16th April	113·4	82·3	104·4	99·7
		F_2 1931-32		
25th February . . .	115·7	71·8	110·9	101·7
		F_3 1931-32		
26th February . . .	121·1	75·5	103·7	99·9

Yield.—Yield in the form of weight of grain per unit area is given in Table II. Although in sowing the spacing was the same in all plots, an acceptable measure of average yield per plant could not be obtained, for mortality, and therefore, effective spacing, was not the same in all four populations [two parents

and two (reciprocal) hybrids). Employing the same notation as for tillering, the significant differences were :

F_1 J × T >T in 1931-32.
>J „ „
F_1 T × J >T in 1931-32.
>J „ „
F_2 J × T >T in 1931-32.
F_2 T × J >T „ „
F_3 No differences significant.
J >T in 1931-32 (in the comparisons of F_1 and F_2 but not of F_3).

TABLE II

Yield of grain (air-dry) per unit area of Joss, Thule, and their F_1, F_2 and F_3 generations. The yields are expressed on the basis of the general mean (of the two parents and the two hybrid generations) = 100.

Year	Joss	Thule	Joss × Thule	Thule × Joss
		F_1		
1929-30	103·9	88·3	104·0	103·7
1931-32	97·5	83·8	111·3	107·4
		F_2		
1930-31	106·6	98·3	94·6	100·0
Duplicate sowing . . .	106·6	95·1	97·7	100·7
1931-32	110·1	81·6	104·1	104·3
		F_3		
1931-32	110·7	95·6	98·9	96·1

In most of the previously published experiments on hybrid vigour the data do not allow of comparison between season and season. There is a noteworthy exception in Bredemann and Heuser [1931] who in a comprehensive six-year experiment with rye were impressed by differences in vigour from year to year. Tables I and II show that for F_1 in 1929-30, Joss × Thule exceeded both parents and its reciprocal in tillering and also in yield, the reciprocal being intermediate between the parents. In 1931-32, however, the F_1's for both directions of the cross exceeded both the parents in tillering and also in yield.

IV. Comparative vigour of the F_1, F_2 and F_3 generations, and of the reciprocal directions, of the cross Little Joss × Thule

For convenience, the reciprocal directions of the cross will be considered first. In the F_1 generation no differences were found in respect of germination and early leaf formation. Constant observation, however, gave rise to the impression that the F_1 J × T was growing, and in particular tillering, far more vigorously than its reciprocal. Periodic counts showed, however, that the tillering difference was significant on only three out of seven occasions in the 1929-30 and 1931-32 crops combined. As Table I shows, the difference had on all seven occasions the same sign, and since the plots were only four times replicated, it may be inferred that the one direction of the cross showed a small but certain increase in vigour of tillering over the other direction. This difference however was not followed up by a difference in yield per unit area. For the F_2 generation there is slight evidence for the superior early leaf formation and tillering of J × T as compared with T × J but no yield difference between them. The reciprocal F_3's showed no significant differences at all.

Taking the F_1, F_2 and F_3 generations together, it is seen that reciprocal differences are fairly common but never large. But it is important to observe that every significant difference is in the same direction, *viz.*, J × T > T × J. Observations made by eye all through the growing period gave rise to the impression that this directional superiority was well marked though far more apparent in the first five or six months than in later life.

The relative vigour of the successive generations in respect of early leaf formation (for germination the data is for F_1's and parents only), tillering and yield indicates that there was a steady decline, *i.e.*, $F_0 < F_1 > F_2 > F_3$.

V. Observations on a wide range of crosses: various phenomena connected with hybridity

The exploratory purpose of a wide range of crosses, each on a scale not large enough for strict statistical examination, has been explained in Section II. The full list of the parents used in the crosses is given in (*4*) of this Section. The main trends of the results will be given in spite of the limitation of statistically low numbers of observations. For, on the one hand, it is difficult to make really adequate numbers of crosses; and on the other, one of the chief clues to the nature of the obscure phenomenon of hybrid vigour is to settle its range of occurrence.

(*1*) *Crossability.*—In hybridising wheats the general impression was obtained that even among closely related forms having the same chromosome number it is easier to cross some varieties than others. Moreover a larger proportion of hybrid grains to florets pollinated was in some cases obtained in one direction of the cross

than in the other. In crosses where the parents mature their anthers at considerably different times, differences in crossability between the reciprocal directions may, perhaps, be simply explained. But in other crosses where no doubt was occasioned by time of anther maturation, certain varieties always proved more successful as female than as male parents.

(2) *Xenia (grain weight effects).*—Table III gives the results from the crosses now under discussion. Their trend suggests that in inter-varietal (*T. vulgare*) crosses, the crossed grain is usually heavier than that of one or other of the parents and often heavier than both. In the other categories of crosses there is a general tendency to decrease below the parental values.

TABLE III

The relative weight of the crossed grain and of the grains of the parent forms. A cross and its reciprocal are separately entered. The crosses were made in 1932. The upper line of each entry in the table gives the number of crosses concerned, the lower (in brackets) the limits of the increase per cent over the stated or the larger-grained parent (F_0) or of the decrease per cent below the smaller-grained parent.

	Crosses between hexaploid wheats (all *T. vulgare*)	Interspecies crosses of tetraploid wheats	Interspecies crosses of hexaploid wheats	Interspecies crosses of tetraploid and hexaploid wheats
Decrease below both F_0's	6 (1-35)	9 (2-47)	4 (8-23)	3 (14-58)
Increase over F_0♂ but not over F_0♀	4 (13-49)	5 (8-20)	7 (1-73)	*Nil*
Increase over F_0♀ but not over F_0♂	11 (2-46)	2 (2-18)	3 (3-36)	*Nil*
Increase over both F_0's	13 (1-24)	2 (1-2)	3 (4-21)	*Nil*
Total crosses made	34	18	17	3

Examination of the results suggests that the occurrence of crossed grains of weight greater than the parental grains is not correlated with the grain size of the male parent.

One of the *vulgare* wheats here used, Tom Thumb, produced characteristically shrivelled grain. The grain it set when pollinated with strongly-growing English varieties of *T. vulgare* were invariably shrivelled and poorly filled. In contrast the *T. vulgare* forms, Chinese White and Iron gave, in all the crosses in which they were the female parent, excellently filled grain.

(3) *Tillering and yield of F_1 and F_0.*—Although actual counts were made as checks upon eye-impressions they cannot be used here because of the smallness of the plant populations concerned. The general conclusion was that most of the F_1's tillered more profusely than their parents, the difference in some cases being very striking indeed. In yield also there appeared to be differences harmonious with those in tillering. The cross, Little Joss × Yeoman, was in two seasons an apparently outstanding example of hybrid vigour in F_1.

(4) *The influence of certain varieties as parents.*—The parents used for this series of crosses were :—

Tetraploid	Hexaploid
Emmer (*T. dicoccum*)	White Spelt (*T. spelta*).
Persian Black (*T. persicum*)	American Club (*T. compactum*).
Kubanka (*T. durum*)	Sphaerococcum (*T. Sphaerococcum*).
Polish (*T. polonicum*)	Red Fife (*T. vulgare*).
Rivet (*T. turgidum*)	Squarehead's Master (*T. vulgare*).
	Little Joss (*T. vulgare*).
	Yeoman (*T. vulgare*).
	Red Marvel (*T. vulgare*).
	Hen Gymro (*T. vulgare*).
	Old Fashioned 2 (*T. vulgare*).
	Tom Thumb (*T. vulgare*).
	Victor (*T. vulgare*).
	Iron (*T. vulgare*).
	Thule (*T. vulgare*).
	Chinese White (*T. vulgare*).

In most cases the cross was made reciprocally and counting a cross and its reciprocal separately, the number of crosses was :—

T. vulgare × *T. vulgare*—56.

Hexaploid species × hexaploid species—17.

Tetraploid species × tetraploid species—18.

Hexaploid species × tetraploid species—3.

It was concluded that some of the varieties influence their F_1 progeny in characteristic ways. The outstanding examples occurred in the *T. vulgare* × *T. vulgare* crosses. Red Marvel, Iron and Old Fashioned 2, whether used as male or female parents, gave in all their crosses, F_1's of markedly lower tillering capacity than the higher tillering of the two parents. Tom Thumb as a female parent, always gave poor F_1's : as male parent it gave very vigorous progeny. The outstanding variety was Chinese White, its F_1's being always very vigorous (for tillering) whether it was used as male or female parent. It was concluded that no general connection subsisted between the vigour of tillering of the parent

varieties and of the resulting F_1. These results reinforce the view of some other investigators [*e. g.*, Jenkins, 1929] that some varieties characteristically give vigorous and others feeble F_1's. Taken with the additional conclusions that some varieties give much better F_1's in one direction than in the reciprocal direction of a cross, it opens an approach, albeit lengthy, to the nature and causes of hybrid vigour.

VI. Physiological aspects

(*1*) *Embryo weight.*—This was studied in the Joss × Thule cross, using ten grains for each parent and for each of the reciprocal directions of the cross, and also in the Joss × Yeoman cross, using 120 grains (12 lots of 10 each) of each of the four categories. The latter cross though not studied in full like the former, appeared to give an exceptionally vigorous F_1. The seeds were placed on layers of filter paper in Petri dishes, just covered with distilled water, and kept for twenty-four hours in an incubator at 23°C. Upon removal the seeds were dried (surface moisture) with filter paper, and weighed. Then the embryos were dissected out and weighed (=fresh weight of embryo) and finally after twenty-four hours in an oven at about 100°C. they were again weighed (=dry weight of embryo). In each case the dry weight of the embryo was the same for crossed grains as that formed by the female parent on normal selfing. The position therefore appears to be different from that in maize where Ashby [1930, 1932] found greater initial weight in hybrid embryos as compared with parental embryos. However Ashby's conclusion that there is no acceleration of growth rate in maize hybrids exhibiting hybrid vigour, the latter phenomenon being attributed to the larger 'capital' with which the hybrid embryo commenced life, has been recently questioned by Lindstrom [1935]. Lindstrom reduced the 'capital' of young hybrid plants by decapitation and found that despite this handicap the hybrids overtook the growth of the untouched parental lines, indicating that the hybrids had a faster growth rate.

(*2*) *The effect of artificial selfing.*—To find out whether the technique of crossing favoured in any way either the selfed grain of the parents or the crossed grain, both naturally and artificially selfed seed of six wheat varieties was obtained under the same growing conditions for all. Artificial selfing was carried out in exactly the same way as artificial crossing, save for the kind of pollen used. It was found that though the rate of germination was more rapid for the artificially selfed seed in the case of five varieties, the total germination was the same for both kinds of selfed seed. The slight "lead" obtained by the former by its earlier germination was not maintained.

(*3*) *Respiration rates of germinating grains, hybrid and parental.*—Measurements of respiration of germinating grains by the Pettenkofer method were very kindly made by Dr. H. R. Barnell. He used the parental and reciprocally crossed grains of the Joss × Thule cross, which as described in Sections III and IV showed

a small but securely demonstrated degree of 'hybrid vigour' in F_1. It was possible to give only ten grains of each of the hybrid seeds. From the results it was possible to infer that up to 180 hours from the time of putting the grains in water the more vigorous parent, Joss, has a decidedly lower respiration rate than Thule; and further, that both kinds of crossed grain respire at about the same rate as Thule.

Experiments were also made on rate of dry weight increase of the growing embryo. The results were not in harmony with either the respiration results or with the idea that hybrid vigour in wheat is controlled, as Ashby concluded it to be in maize, by initial embryo weight.

(4) *Some general inferences.*—An examination was made of the whole of the literature bearing upon hybrid vigour and a list was assembled of all the crosses in which the phenomenon appears to have been clearly shown. Admissibility of evidence has had to be determined from the recorded circumstances, for the cases are few indeed in which trustworthy numerical evidence is given. Of the apparently sound cases fourteen are for crosses among varieties of the same species, the species involved being: *Zea Mays*, *Z. saccharata*, *Andropogon Sorghum*, *Secale cereale*, *Solanum melongena*, *Lycopersicum esculentum*, *Petunia violacea*, *Digitalis purpurea*, *Minulus luteus*, *Viola tricolor*, *Dianthus caryophyllus*, *Nemophila insignis*, *Brassica oleracea*, *Soja max*.

Of interspecies crosses in which hybrid vigour seems to have been shown clearly there are twenty-nine cases involving two or more species of the following genera: *Solanum*, *Datura*, *Petunia*, *Lycium*, *Nicotiana*, *Verbascum*, *Digitalis*, *Pentstemon*, *Malva*, *Althaea*, *Lavatera*, *Tropaeolum*, *Cucumis*, *Lobelia*, *Mirabilis*.

So far as this list goes there is evidence that hybrid vigour occurs preponderantly in crosses among parents which are (*a*) diploid, (*b*) naturally out-pollinating, (*c*) endospermic in seed. The significance of the association of these with hybrid vigour cannot yet be judged.

VII. Summary

An intensive investigation of hybrid vigour in the cross Little Joss × Thule (both varieties of *T. vulgare*) in the F_1, F_2, and F_3 generations was made. In addition a range of ninety-four crosses was studied on a small scale; of these fifty-six were between varieties of *T. vulgare*, the remainder being interspecies, some with parents of the same chromosome number and some not. "Vigour" variously specified by investigators, was here taken to be yield of grain together with the associated attribute, tillering. Certain physiological experiments were also made on grain weight, germination rate, embryo weight and respiration rate of the germinating grain. The crossses were made reciprocally.

Clear evidence of hybrid vigour was displayed in the cross Little Joss × Thule, though the manifestation was not so striking as, for example, in maize.

Between the reciprocal directions of the cross, small but statistically certain differences were established in F_1: in F_2 there were slight indications of difference, the F_3's were identical in vigour.

Vigour in the successive hybrid generations as compared with the parents (F_0) was clearly in the order $F_0 < F_1 > F_2 > F_3$.

From the wide range of crosses a number of general inferences was drawn (1) In some varieties a restricted optimum period for crossing was found. (2) Certain wheats regularly proved more successful (proportion of successful pollinations) as female than as male parents. (3) In crosses of *T .vulgare* × *T. vulgare* the actual crossed grain was in most cases of greater weight than that of one parent and often heavier than either. With interspecies crosses, however, even when the parents had the same chromosome number, decrease instead of increase was found in most cases. (4) An F_1 of greater tillering capacity and yield than its parents resulted from most of the crosses. (5) But there were important exceptions and certain varieties stood out as good or bad, in respect of vigour of F_1 progeny, when used as male parent or female or in both directions of crossing.

From the physiological experiments it was clear that Ashby's explanation of hybrid vigour in maize F_1's as resulting solely from the greater embryo weight of the crossed grain, was inapplicable to wheat. No increase in weight over parental embryo weight occurred. Measurements of respiration rate gave further evidence against the possibility that embryo weight influences hybrid vigour in wheat.

From examination of all the published cases of hybrid vigour it appears that the phenomenon occurs preponderantly in crosses among parents which are (*a*) diploid, (*b*) naturally out-pollinating and (*c*) endospermic in seed.

REFERENCES

Ashby, E. (1930). *Ann. Bot.* **44**, 457.
——— (1932). *Ann. Bot.* **96**, 1007.
Bredemann, G., and Heuser, W. (1931). *Zeit. Zuchtung* **A. 16**, 1.
Jenkins, M. T. (1929). *J. Agric. Res.* **39**, 677.
Jones, D. F. (1918). *Cornell Agric. Expt. Stn. Bull.* No. 207.
Lindstrom, E. W. (1935). *Amer. Nat.* **69**, 68.

A NOTE ON THE CLASSIFICATION OF INDIAN SAFFLOWER

BY

T. S. SABNIS, B.A., M.Sc., I.A.S.,

Economic Botanist to Government, United Provinces,

AND

M. G. PHATAK, L.Ag. (Bom.),

Research Assistant

(Received for publication on 8th August 1935)

CONTENTS

I. Introduction

Excepting the pioneer work of Howard, Howard and Khan [1915] and the supplementary note by Khan [1929], there is no other literature available on the classification of Indian safflower (*Carthamus tinctorius* L.). Both these works were found helpful in the isolation and identification of the types by the writers, during the course of their investigation on this crop.

While studying the botanical characters of the safflower types the writers, however, felt that some of the characters used by Howard, Howard and Khan in their classification were not sufficiently clear. Their classification was based on rather broad divisions of the plant characters.

For example, the spinose and spineless types are grouped in one common class II " Outer bracts ovate to rounded, moderately spinose to spineless ", of the key. Similarly the Types 9, 15, 16, 18 and 21 with lanceolate and spinose bracts and the Types 29, 30, 31, 32, 33 and 34 with lanceolate and spineless bracts, as mentioned in the description of these types, have also been included in this class. Again, such characters as (1) ' moderately spinose ', (2) faint red dot at the apex in Types 28 and 29, (3) trace of red near the apex in Type 14, and (4) presence of red streaks on the sides of the flower buds in Types 29, 30, which are not sufficiently clear, are used in the key. Such characters are therefore likely to be misused. It was

observed that red colour in the form of red streaks or red splashing, noticed in the flower buds of some of the types, was probably due to aphis attack, although these types were normally free from such red colouration.

Besides, the twenty-nine new types which were discovered by the writers during the course of their investigations could not be fitted in the classification of Howard Howard and Khan.

The revision of the classification of Indian safflower is therefore attempted by the writers, using the classification of Howard, Howard and Khan as its basis. In their revised classification single and distinctive characters are used, indefinite terms are as far as possible avoided, and its scope is enlarged by the inclusion of the twenty-nine new types.

The original numbers of the Pusa types have been retained and supplementary characters of diagnostic importance are noted under each. The new types have their diagnostic characters described in detail. Owing to the re-distribution of the Pusa types and retention of their original numbers serial order of the types could not be kept either in the key or in the description of the types.

Finally the writers express their indebtedness to Dr. F. J. F. Shaw, Imperial Economic Botanist, for his helpful suggestions.

II. Key to the types

1. Bracts spinose

(1) Outer bracts lanceolate.

(A) Inner bracts smooth and green.

(*a*) Florets white.

Plants erect—Type 1.

Plants spreading—Types *35*, *36*, *37*.

(*b*) Florets yellow, turning to brownish yellow on fading.

Plants erect—Type *38*.

Plants spreading—Types 2, 3, 4.

(*c*) Florets yellow, turning to red on fading.

Plants erect—Types 25, *39*, *40*, *41*, *42*.

Plants spreading—Types 5, 6, 7, 20, 21, 23, 26, 27, 28, *43*, *44*, *45*, *46*, *47*, *48*.

(*d*) Florets reddish orange, turning to red on fading.

Plants erect—Type *49*.

N.B.—The types in Italics are new ; the rest are Pusa types.

(B) Inner bracts felted and white.

(*a*) Florets white.

Plants erect—Type *50*.

(*b*) Florets yellow, turning to brownish yellow on fading.

Plants erect—Type 9.
Plants spreading—Types *51*, *52*.

(*c*) Florets yellow, turning to red on fading.

Plants erect—Type *53*.
Plants spreading—Types 15, 16, *54*.

(*d*) Florets deep red.
Plants erect—Type *55*.

(2) Outer bracts obovate to rounded.

(A) Inner bracts smooth and green.

(*a*) Florets yellow, turning to red on fading.

Plants erect—Types 19, 22.
Plants spreading—Types *56*, *57*.

(*b*) Florets deep red.

Plants erect—Type 24.
Plants spreading—Type *58*.

(B) Inner bracts felted and white.

(*a*) Florets white.

Plants erect—Type *59*.

(*b*) Florets yellow, turning to brownish yellow on fading.
Plants spreading—Type 8.

(*c*) Florets yellow, turning to red on fading.
Plants erect—Types 10, 12, 13, 14, *60*.

2. Bracts spineless

(1) Outer bracts lanceolate.

(A) Inner bracts smooth and green.

(*a*) Florets orange yellow, turning to red on fading.
Plants erect—Types 29, 30.
Plants spreading—Types 31, 32, 33, 34.

(2) Outer bracts obovate to rounded.

(A) Inner bracts smooth and green.

(*a*) Florets yellow, turning to orange red on fading.

Plants erect—Type 18.

(B) Inner bracts felted and white.

(*a*) Florets white.

Plants erect—Type *61*.

(*b*) Florets yellow, turning to red on fading.

Plants erect—Types 11, *62*.

(*c*) Florets deep red.

Plants erect—Type 17.

Plants spreading—Type *63*.

III. Descriptions of the types*

I. Bracts spinose, outer lanceolate, inner smooth and green

(*a*) *Florets white*

Type 1.—Plants tall, above 130 cm. in height. Bracts with apical spines and without hairs. Maturity late.

Type 35.—Habit spreading. Plants intermediate in height, about 115 cm. high. Leaves dark green, deeply incised, 18-21×4·5-5 cm. Bracts leafy, lanceolate, spiny. Florets white, changing to dirty white. Maturity late.

Type 36.—Habit spreading. Plants short, less than 95 cm. in height. Leaves dark green, slightly incised or sometimes dentate, 16-19 × 4·5-5 cm. Bracts and florets as in Type 35. Maturity late, but earlier than in Type 35.

Type 37.—Habit spreading. Plants intermediate in height, about 105 cm. high. Leaves incised, darker than in Type 36, 17-19×4-4·5 cm. Bracts as in Type 36 but narrower and shorter. Florets as in Type 35. Maturity late.

(*b*) *Florets yellow, changing to brownish yellow on fading*

Type 2.—Plants intermediate in height, about 115 cm. high. Leaves slightly incised or deeply dentate. Maturity intermediate.

Type 3.—Plants intermediate in height, slightly taller than in Type 2. Maturity early.

*Plants up to 100 cm. high are termed short, 100-130 cm. intermediate and above 130 cm. tall. Plants maturing within 150 days are termed early, those maturing within 150-160 days intermediate and those maturing after 160 days late. The descriptive notes on the Pusa types have been confined to supplementary characters of diagnostic importance.

Type4.—Plants short, about 95 cm. high.

Type 38.—Habit erect. Plants tall, height 130 cm. Leaves light green, with few short marginal spines, serrate, 15-18 × 3-5 cm. Bracts lanceolate, green, spinose with long spines. Florets very light yellow, turning to light red on fading. Maturity late.

(c) *Florets yellow, turning to red on fading*

Type 5.—Plants intermediate in height, about 115 cm. high. Maturity early.

Type 6.—Plants tall, about 145 cm. in height. Leaves light green, serrate to dentate. Maturity very early.

Type 7.—Plants tall, about 135 cm. high. Leaves light green, lighter than in Type 6, serrate to dentate. Maturity early.

Type 18.—Plants tall, about 145 cm. in height. Bracts slightly spiny. Florets yellow, turning to orange red on fading. Maturity late.

Type 20.—Plants tall, erect and compact, between 100 and 140 cm. high. Maturity early.

Type 21.—Plants intermediate in height, about 125 cm. high. Leaves serrate to dentate. Florets yellow, turning to orange red on fading. Maturity intermediate.

Type 23.—Plants intermediate in height, about 105 cm. high. Bracts lanceolate to elliptical, green and slightly spiny. Maturity intermediate.

Type 25.—Habit somewhat erect or tending to be spreading. Plants intermediate in height, about 115 cm. high. Maturity early.

Type 26.—Plants very short, about 80 cm. hlgh. Maturity early.

Type 27.—Plants short, about 100 cm. high. Bracts, flower buds and florets as in Type 26, but the red dot at the apex of the flower buds less distinct. Maturity early.

Type 28.—Plants intermediate in height, about 115 cm. high. Florets yellow, turning to orange red on fading. Maturity early.

Type 39.—Habit erect. Plants intermediate in height, about 125 cm. high. Leaves light green, incised, 24-27 × 6·5-7 cm. Bracts lanceolate to elliptical, rarely rounded, smooth, green and slightly spinose. Flower buds yellow with red base and red tip. Florets yellow, changing to red on fading. Maturity late.

Type 40.—Habit erect. Plants tall, 100-130 cm. in height. Leaves dark green, broadly dentate. Bracts lanceolate, long, green, smooth and spinose. Flower buds yellow. Florets yellow, turning to red on fading. Maturity late.

Type 41.—Habit erect. Plants tall, 100-140 cm. in height. Leaves light green serrate. Bracts lanceolate, long, green, and smooth and spinose. Flower buds yellow. Florets yellow, turning to red on fading. Maturity very late.

Type 42.—Habit erect. Plants tall, 100-140 cm. in height. Leaves very large, incised, green. Outer bracts lanceolate with a few short spines. Inner bracts obovate to rounded, green, smooth, with a few short spines. Flower buds deep yellow. Florets deep yellow, turning to red on fading. Maturity late.

Type 43.—Habit spreading. Plants short, about 95 cm. in height. Leaves light green and serrate, 14-16 × 4-5 cm. Outer bracts lanceolate, very long and with long spines. Flower buds yellow with reddish splashing on the sides and tips yellow without red dot at the apex. Florets yellow, fading to orange red. Maturity early.

Type 44.—Habit spreading. Plants short, 80-100 cm. high. Leaves green, broadly dentate, 18-22 × 6-6·5. Bracts lanceolate, short but broad, and green. Flower buds yellow, turning to red on fading. Maturity intermediate.

Type 45.—Habit spreading. Plants intermediate in height, about 105 cm. high. Leaves light green, broadly dentate, 17-22 × 5-6 cm. Bracts—outer lanceolate to elliptical, and inner lanceolate or obovate to rounded, green and spinose. Florets yellow, changing to red on fading. Maturity late.

Type 46.—Habit spreading. Plants short, about 95 cm. high. Leaves light green and serrate, 17-18·5×4·5-5·5 cm. Bracts as in Type 23. Flower buds with distinct red base and yellow tip. Florets reddish yellow or orange from the start and red on fading. Maturity intermediate.

Type 47.—Habit spreading. Plants short, about 100 cm. in height. Leaves green, dentate to serrate. Bracts lanceolate, smooth, green and spiny, 14-15 × 3·5-4 cm. Flower buds yellow with red splashing at the base and red towards the tip with yellow apical dot. Florets reddish yellow, changing to red on fading. Maturity intermediate.

Type 48.—Habit spreading. Plants short, about 85 cm. high. Leaves light green, serrate or slightly dentate, 14-16 × 4·5. Bracts lanceolate, green and spinose. Flower buds with distinct red base, reddish below the apex and with no red dot at the apex. Florets yellow, turning to red on fading. Maturity intermediate.

(*d*) *Florets reddish orange, changing to red on fading*

Type 49.—Habit erect. Plants intermediate in height, about 115 cm. high. Leaves green, deeply incised, 26-35 × 6-13 cm. Bracts lanceolate, rarely obovate to rounded, smooth, green and slightly spinose. Flower buds reddish with yellow tips. Florets reddish orange, changing to red on fading. Maturity late.

II. Bracts spinose, outer lanceolate, inner felted and white

(*a*) *Florets white*

Type 50.—Habit erect. Plants intermediate in height, about 125 cm. high. Leaves dark green, incised, 20-26×4·5-5·5. Bracts lanceolate, felted and white, and moderately spinose with short spines. Flower buds white. Florets white, changing to dirty white on fading. Maturity intermediate.

(*b*) *Florets yellow, turning to brownish yellow on fading*

Type 9.—Habit erect. Plants tall, about 145 cm. high. Leaves dark green, dentate to incised. Bracts lanceolate, with a few short spines, felted and white. Maturity late.

Type 51.—Habit spreading. Plants short, about 100 cm. high. Leaves green and broadly dentate, 20-23·5 × 4·5-5·5. Bracts lanceolate, long, felted and white, and slightly spinose. Flower buds pale yellow. Florets yellow, turning to brownish yellow on fading. Maturity early.

Type 52.—Similar to Type 51 in habit, height, flower buds, florets and maturity. Leaves broadly serrate, 16-18 × 4·5-5. Bracts, however, are distinctly shorter and narrower than in Type 51, though lanceolate, felted and white, and slightly spinose as in Type 51.

(*c*) *Florets yellow, turning to red on fading*

Type 15.—Plants short, about 100 cm. high. Maturity intermediate.

Type 16.—Plants intermediate in height, about 110 cm. high. Maturity intermediate.

Type 53.—Habit erect. Plants intermediate in height, about 120 cm. high. Leaves light green, incised, 23-28 × 5-7·5 cm. Bracts lanceolate, felted and white, and spinose with apical spine prominent. Flower buds yellow with no red dot at the apex. Florets yellow, turning to orange red on fading. Maturity late.

Type 54.—Habit spreading. Plants intermediate in height, about 110 cm. high. Leaves green and incised, 17-20 × 4·5-5 cm. Bracts lanceolate to elliptical, felted and white, and spinose. Flower buds yellow with red dot at the apex. Florets yellow, turning to red on fading. Maturity intermediate.

(*d*) *Florets deep red*

Type 55.—Habit erect. Plants intermediate in height, about 115 cm. high. Leaves dark green, incised, 20-30 × 6-8 cm. Bracts lanceolate to elliptical, felted and white, and with a few short spines. Flower buds deep red with yellow apical dot. Florets orange red, fading to deep red. Maturity late.

III. Bracts spinose, outer obovate to rounded, inner smooth and green

(*a*) *Florets yellow, turning to red on fading*

Type 19.—Plants tall, about 165 cm. in height. Bracts obovate to rounded, smooth and green, with a few short spines. Maturity late.

Type 22.—Habit erect. Plants tall, height a little over 130 cm. Leaves serrate to dentate. Maturity early.

Type 56.—Habit spreading. Plants intermediate in height, about 115 cm. high. Leaves green, serrate, but not spinose, 16-22 × 5-6 cm. Bracts obovate to rounded, spinose, smooth and green. Flower buds yellow with no red dot at the apex. Florets yellow, turning to red on fading. Maturity intermediate.

Type 57.—Habit spreading. Plants short, about 100 cm. in height. Leaves green, serrate but not spinose, 13-15 × 3-4 cm. Bracts obovate to rounded, smooth and green, and with very short spines. Flower buds, florets and maturity as in Type 56.

(*b*) *Florets deep red*

Type 24.—Plants tall, about 150 cm. in height. Bracts obovate to rounded. Maturity intermediate.

Type 58.—Habit spreading. Plants intermediate in height, about 110 cm. high. Leaves dark green, incised, 14-16 × 4-4·5 cm. Bracts obovate to rounded, smooth and green and slightly spinose. Inner bracts very small. Flower buds deep red with a yellow apical point. Florets orange red, turning to deep red. Maturity intermediate.

IV. Bracts spinose, outer obovate to rounded, inner felted and white

(*a*) *Florets white*

Type 59.—Habit erect. Plants tall, about 145 cm. in height. Leaves light green, broadly serrate, 14-19 × 3·5-5·5 cm. Bracts obovate to rounded, felted and white, and slightly spinose. Flower buds and florets white, changing to dirty white. Maturity early.

(*b*) *Florets yellow, turning to brownish yellow on fading*

Type 8.—Plants intermediate in height, about 115 cm. high. Bracts obovate to rounded, slightly spinose. Maturity intermediate.

(*c*) *Florets yellow, turning to red on fading*

Type 10.—Plants tall, about 100 cm. high. Leaves broadly serrate at the upper end and slightly incised at the lower. Bracts quite broad, obovate to rounded, slightly spinose. Maturity late.

Type 12.—Plants intermediate in height, about 120 cm. high. Leaves green, serrate and slightly incised at places towards the base. Bracts obovate to rounded, slightly spinose. Inner bracts very short. Maturity intermediate.

Type 13.—Habit erect. Plants tall, about 155 cm. in height. Leaves light green, dentate towards the top and incised towards the base. Maturity late.

Type 14.—Plants tall, about 150 cm. high. Bracts obovate to rounded or elliptical to rounded, slightly spinose. Flower buds deep yellow with a red dot at the apex. Maturity intermediate.

Type 60.—Habit erect. Plants intermediate in height, about 110 cm. high. Leaves dark green, serrate, 18-25 × 6-7 cm. Bracts obovate to rounded, very large in size, felted and white, and with a few short spines. Flower buds yellow with no red dot at the apex. Florets yellow, turning to red on fading. Maturity intermediate.

V. BRACTS SPINELESS, OUTER LANCEOLATE, INNER SMOOTH AND GREEN

(*a*) *Florets yellow, turning to red on fading*

Type 29.—Plants intermediate in height, about 105 cm. high. Flower buds deep orange yellow, with an apical red dot absent or very faint, but with distinct red streaks or red splashing on the sides. Maturity early.

Type 30.—Plants intermediate in height, about 125 cm. high. Leaves serrate. Flower buds deep orange yellow with an apical red dot very faint or absent. Maturity intermediate.

Type 31.—Plants short, about 95 cm. in height. Flower buds deep orange yellow with an apical red dot very faint or absent, but with red streaks on the sides. Similar to Type 29, except in the habit and height. Maturity intermediate.

Type 32.—Plants short, about 100 cm. high. Flower buds orange yellow with distinct red streaks on the sides. Maturity early.

Type 33.—Plants short, about 100 cm. in height. Flower buds orange yellow with distinct red streaks. Maturity early.

Type 34.—Plants short, about 100 cm. in height. Flower buds deep orange yellow, with distinct red streaks. Maturity intermediate.

VI. BRACTS SPINELESS, OBOVATE TO ROUNDED, INNER FELTED AND WHITE

(*a*) *Florets white*

Type 61.—Habit erect. Plants intermediate in height, about 120 cm. high. Leaves dark green, incised, 16-19 × 4·5-6 cm. Bracts obovate to rounded, felted and white, comparatively very few in number and very small in size, and spineless. Flower buds and florets white, turning to dirty white on fading. Maturity early.

(*b*) *Florets yellow, turning to red on fading*

Type 11.—Plants tall, about 145 cm. high. Leaves dark green, serrate. Bracts obovate to rounded and spineless. Maturity intermediate.

Type 62.—Habit erect. Plants intermediate in height, about 120 cm. high. Leaves green, incised, 25-30 × 6-7·5 cm. Bracts elliptical or obovate to rounded, felted and white, and spineless. Flower buds yellow, with no apical red dot. Florets yellow, turning to orange red on fading. Maturity late.

(c) *Florets deep red*

Type 17.—Plants tall, about 165 cm. high. Bracts obovate to rounded. Maturity intermediate.

Type 63.—Habit spreading. Plants intermediate in height, about 110 cm. high. Leaves green, incised, 20-25 × 5-6 cm. Bracts obovate to rounded, felted and white, spineless. Flower buds deep red, with yellow tips. Florets orange red, turning to deep red on fading. Maturity intermediate.

Summary

1. The work of the previous investigators on Indian safflower (*Carthamus tinctorius* L.) has been reviewed.

2. The crop as a whole has been classified into types on the basis of distinctive characters.

3. A key to the types is worked out.

4. Twenty-nine new types have been added with short diagnostic notes on each.

REFERENCES

Howard, A.; Howard, G. L. C. and Khan, A. R. (1915). *Mem. Dept. Agric. Ind., Bot. Ser.* **7**, 237-255.

Khan, A. R. (1929). *Mem. Dept. Agric. Ind., Bot. Ser.* **18**, 81-87.

INFLUENCE OF STEEPING IN SOLUTIONS OF VARYING HYDROGEN-ION CONCENTRATIONS ON THE GERMINATION AND EARLY GROWTH OF CANES

BY

B. N. SINGH, D.Sc.,

Kapurthala Professor of Plant Physiology and Agricultural Botany, Director, Institute of Agricultural Research, Benares Hindu University,

S. C. CHAKRAVARTI, M.Sc.,

AND

Y. V. RAO, M.Sc.

(Contributions from the Institute of Agricultural Research, Benares Hindu University)

(Received for publication on 23rd July 1935)

(With four text-figures)

Introduction

The question of increasing the growth and sucrose content of canes has been engaging the attention of scientific agriculturists since a long time and a good many measures have been advocated towards the initiation of a profitable sugarcane cultivation. One of the most common practices in this direction is the pre-soaking of canes in water under completely submerged conditions for varying durations of time which not unoften results in increased and rapid germination of the buds followed by an early establishment of the plant.

The experiments performed during the previous years on the physiology and chemistry of sugarcane at this Experiment Station gave encouraging results in this regard, and conclusive evidence was forthcoming in favour of the possibilities of increasing the sucrose content and growth of canes. Among the many treatments which accelerate germination and growth, the soaking of canes in solutions of varying hydrogen-ion concentrations has been one. To the elucidation of the results obtained in this connection the present paper is mainly devoted.

The cane variety used for experimentation is the well-known Co. 331 which has been fully adapted to the conditions of the experimental farms at this Experiment

Station for a number of years. The choice fell on this variety of cane as it provided a tall, erect stem of more or less uniform diameter having long internodes. Canes of the same age and developmental stage are selected and discarded to the extent of one-third each way while the remaining one-third middle portion of more or less uniform sugar content is selected for experimentation. From this are cut setts of uniform diameters and fresh weight, eight inches in length with a single eye in the middle. Special care is taken to discard injured setts and eyes, as well as to standardise as far as possible the size and condition of the buds so that any difference on this account might not vitiate subsequent analysis of the data.

The variation in the pH value is brought about by the use of Merck's normal hydrochloric acid and caustic soda solutions which are diluted to yield the desired pH. Following are the concentrations used with their respective pH values :—

Hydrochloric acid		Sodium hydroxide	
N/100	pH 2	*N*/100	pH 12
N/10,000	pH 4	*N*/10,000	pH 10
N/1,000,000	pH 6	*N*/1,000,000	pH 8

For varying the hydrogen-ion concentration buffer solutions could not be used, as all of them are composed of a number of different chemicals. In consequence it becomes difficult to judge whether the observed effect is due to the alteration in the hydrogen-ion concentration or to the changed concentration of the salts used. As the reaction alters after a certain period of contact between the solutions and cane setts, the liquid is frequently changed. The temperature during steeping is maintained exactly at 15°C.

After the allotted period of twenty-four, forty-eight and ninety-six hours the setts are taken out of the solution, thoroughly rinsed with tap water and allowed to grow in earthenware vessels after Venkatraman [1924] with necessary modifications, the nutrient media used being water. The control sett direct from the field is also allowed to grow side by side in the same manner.

The germination rate of both sett roots and buds as well as their sugar content are recorded. The germinated seedlings are harvested after a period of two-and-half months after sowing and their final dry weight is determined. An average of a large number of canes from each pH is used to allow the biometrical treatment of results. Sugar is determined at three distinct stages in the life-cycle (*i*) when the sett roots sprout, (*ii*) when the buds germinate, and (*iii*) at harvest. A random sample of ten setts is taken out, 1½ inches from both the ends rejected and the rest crushed to get the juice. This is done in duplicate and in setts where the difference between the two samples is significant, analysis is extended to fresh ones.

The modified picric acid method is adopted for the determination of the reducing sugars and sucrose [Willaman and Davison, 1924]. Calculation is made according to the corrected table given by these authors.

Experimental results

A. Germination energy of sett roots and buds

Three criteria have been adopted for determining the best germination energy. They are (*a*) early start, (*b*) early attainment of a high maximum and (*c*) early completion of the process. A study of the curves (Figs. 1 and 2) representing the percentage of setts showing germination on the various dates indicates that the differently trea ted setts differ in all these respects.

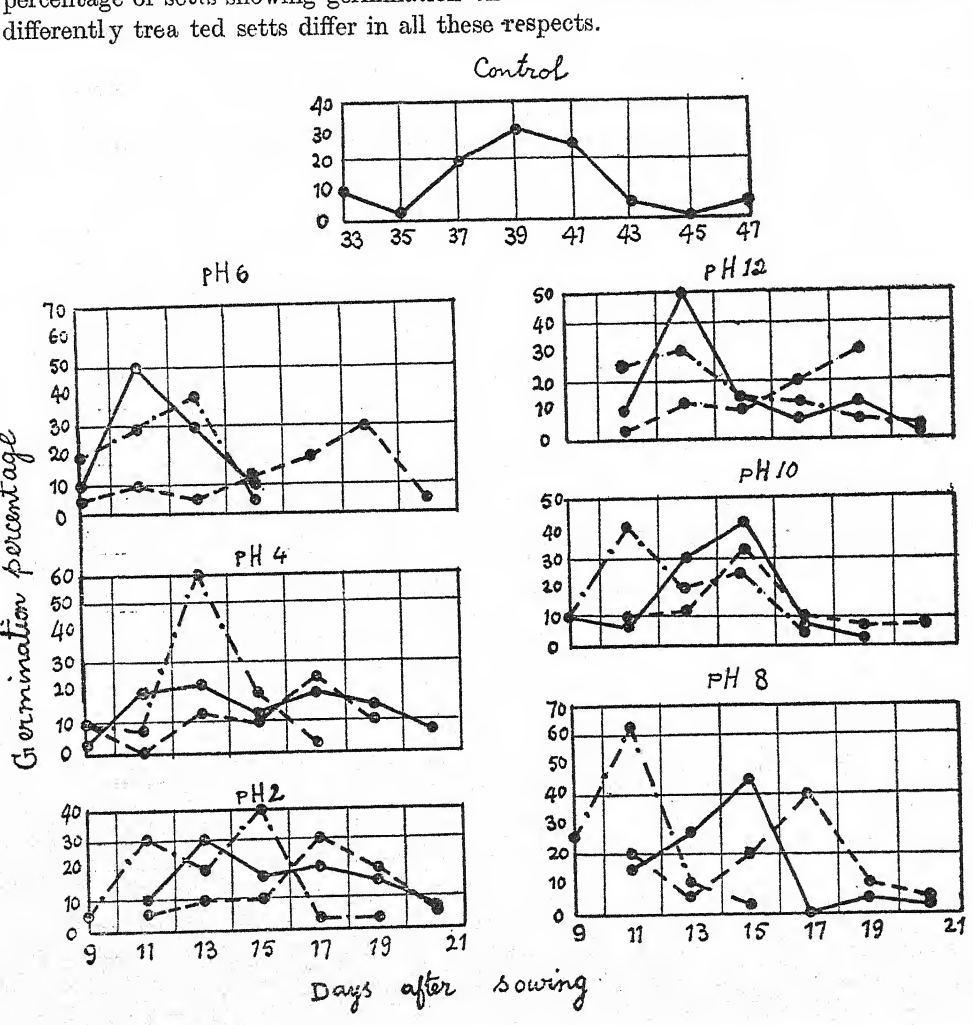


Fig. 1. The germination energy of sett roots in the differently treated setts.

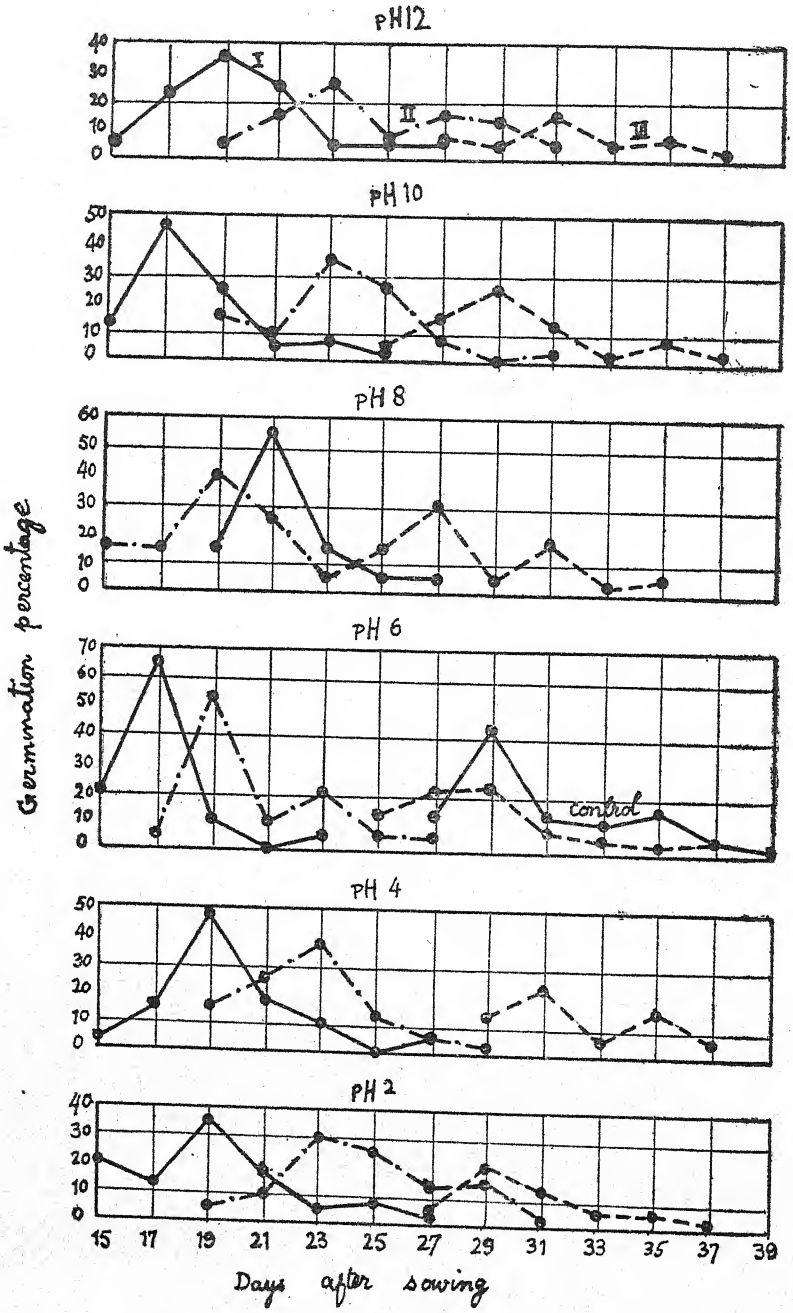


Fig. 2. Germination energy of buds in the differently treated setts. I 24 hours. II 48 hours. III 96 hours.

Germination of sett roots in some of the treated canes starts nine days after sowing and extends in certain cases up to the twenty-first day. A comparative study of the rate of germination in the light of the above-mentioned criterion for the different periods of treatment, *i.e.*, twenty-four hours, forty-eight hours and ninety-six hours indicates that the canes treated for forty-eight hours germinate earliest on the ninth day in five hydrogen-ion concentrations out of six, the exception being at the extreme alkaline side. Next to this come setts treated for twenty-four hours. The superiority of forty-eight hour treatment is also indicated by the early attainment of a high maximum. Setts treated at pH 8 and 10 attain the maximum on the third day after the start; the former comes to a value of 62·5 per cent, the highest record on a single day, while the value for the latter is only 40 per cent. Maximum on the 5th day after the start is shown by the setts treated at pH 4, 6 and 12, the values attained being 62·5, 40, and 30 per cent respectively. From this point of view also ninety-six hour treatment comes last with the highest maximum of 32·5 per cent in pH 8. As to the rapidity of finishing the process, the twenty-four hour and ninety-six hour periods decidedly lag behind the forty-eight hour, as in the majority of the cases the process comes to an end as early as the thirteenth day.

As to the comparative effect of the different hydrogen-ion concentrations the best germination energy is found to be displayed in all the three periods by the canes treated in hydrogen-ion concentrations near about neutrality. Thus for the forty-eight hour period the highest maximum of 62·5 per cent is shown by pH 8. The same for twenty-four hour period is noticed at pH 6.

A cent per cent germination occurs in the twenty-four and forty-eight hour periods. The germination percentage in the ninety-six hour period is low in comparison to the previous ones which clearly indicates the harmful effect of this prolonged period of treatment. Out of the total canes in each case in pH 2—80 per cent, pH 4—67·5 per cent, pH 6—87·5 per cent, pH 8—97·5 per cent, and pH 12—77·5 per cent showed germination.

The control starts with a value of four as late as thirty-four days after sowing and its germination energy is also poor with a maximum of only thirty per cent towards the middle. It is interesting to note in this connection that germination of sett roots in all the treated ones occurs twenty-four days prior to the control.

In the case of buds the germination starts one week after the sprouting of sett roots. Unlike the sett roots the best germination energy is displayed by setts treated for twenty-four hours. In this period out of six hydrogen-ion concentrations five indicate sprouting on the seventeenth day. In the forty-eight hour period of treatment only pH 6 sprouts on the third day while the rest are delayed up to the nineteenth (pH 8) and the twenty-first days. The bud germination in ninety-six hour period of treatment closely corresponds to the sett root germination and indicates the deleterious effect of this prolonged period of soaking. The germination starts very late and in certain cases occurs

only two days prior to the completion of this process in the setts of the previous treatments. The maximum in twenty-four hour period comes earliest and occurs on the nineteenth day at pH 6 and 10 with values of 70 and 47·5 per cent respectively. In ninety-six hour period the maximum is extremely low, the highest being in pH 8 with a value of 32·5 per cent only. As to the rapidity of finishing the process, the forty-eight hour and ninety-six hour periods decidedly lag behind. Out of six cases, in five the twenty-four hour period completes earlier than the forty-eight hour. The deleterious effect of the ninety-six hour period of treatment is also shown by the low percentage of total germination. Both the extremes are harmful but the extreme alkaline side is more deleterious in effect than the extreme acidic side. This becomes more clear when the total number of setts germinated at each treatment is noted. They are as follows :—pH 2—50 per cent, pH 4—60 per cent, pH 6—75 per cent, pH 8—80 per cent, pH 10—62·5 per cent and pH 12—42·5 per cent. Best results are found to occur at pH 6 and 8 and are similar to those noted for roots.

The control gives a cent per cent germination. Sprouting begins on the thirtieth day and a maximum of 42·5 per cent is reached two days later. The process stops on the forty-second day.

B. Sugar content of the mother sett

The curves for sucrose and reducing sugars (Fig. 3) indicate that in all the three periods the sucrose value falls from first to the last stage. The widest fall is noticed in the setts treated for twenty-four hours and is followed by those treated for forty-eight and ninety-six hours, respectively. As to the relative degree of the sucrose conversion among the variously treated setts a close examination of the curves will reveal that in the majority of cases they possess a depression in the middle, that is, near about the neutral point. Except the first stage in ninety-six hour period the alkaline extreme shows a greater presence of sucrose than the extreme acidic side.

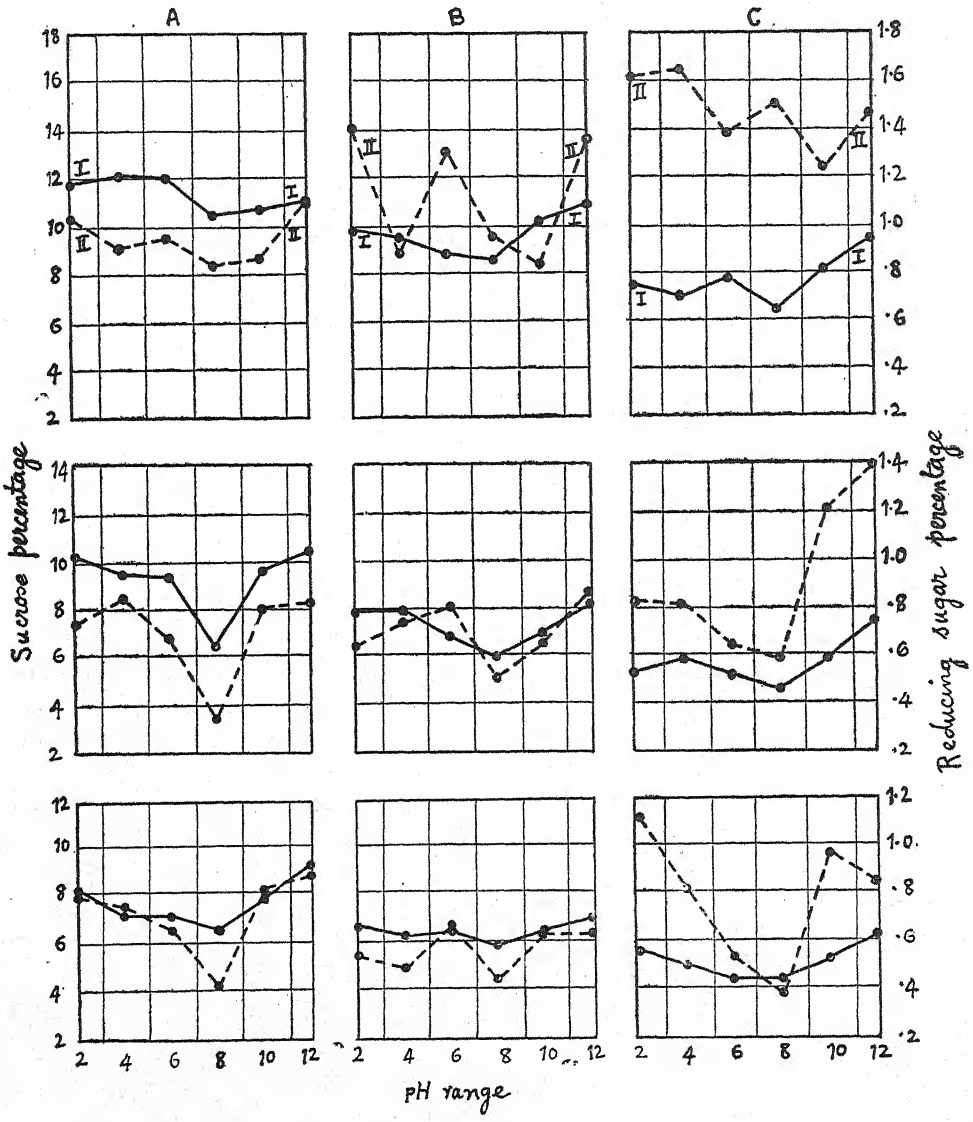


Fig. 3. The values of sucrose and reducing sugar contents of the differently treated setts during A—bud germination stage. B—sett-root germination stage and C—harvest stage. I Sucrose. II Reducing sugar. Top row—96 hrs. treatment; middle row—48 hrs. treatment; and bottom row—24 hrs. treatment.

The values obtained for glucose in majority of the cases run parallel to the sucrose and shows a minimum value where there is less sucrose. As to their production there is not much difference between twenty-four hour and forty-eight hour periods but the ninety-six hour period stands distinctly from them.

Here for all the stages the reducing sugars show a clear accumulation. A high accumulation of glucose is also noted in the third stage of all the different periods of treatment in both extreme acid and alkaline sides.

The rate of conversion of sucrose is more rapid when the period of treatment is short. There is not much regularity about the glucose but a study of the longer-treated setts (96 hours) indicates that a higher accumulation of this substance may be due to a long period of treatment.

The sucrose consumption is more in setts treated with neutral solutions than the setts treated with either higher or lower pH. The reducing sugars also, to a certain extent, run parallel to the sucrose and there seems to be a relatively high consumption of this substance in setts treated in solution which were near about neutrality. In treatments ranging over a very long period a greater accumulation of sugar is noticed towards the extreme acidic and alkaline sides.

C. Production of dry matter

Roots.—The beneficial effect of soaking becomes quite evident when we study the dry matter production. A reference to Fig. 4 indicates that except pH 4 of ninety-six hour treatment all others give a better dry matter output than the control. Of the three periods, forty-eight hour series show a marked superiority to the rest, while the ninety-six hour sett occupies the lowest position as usual. The dry weight increases from the extreme acid side, attains a maximum at pH 8 and then comes down up to the end. A fall is noticed at pH 6 of the shorter period. The nature of the curve in twenty-four hour period is different from others. Here the readings start with a low value, increase up to pH 6, after which they remain more or less constant up to pH 12. The decrease in the output of dry matter towards the acid side does not appear to be due to an over-dose as a prolongation of this period to forty-eight hours appears to be beneficial as indicated by an increase in dry weight.

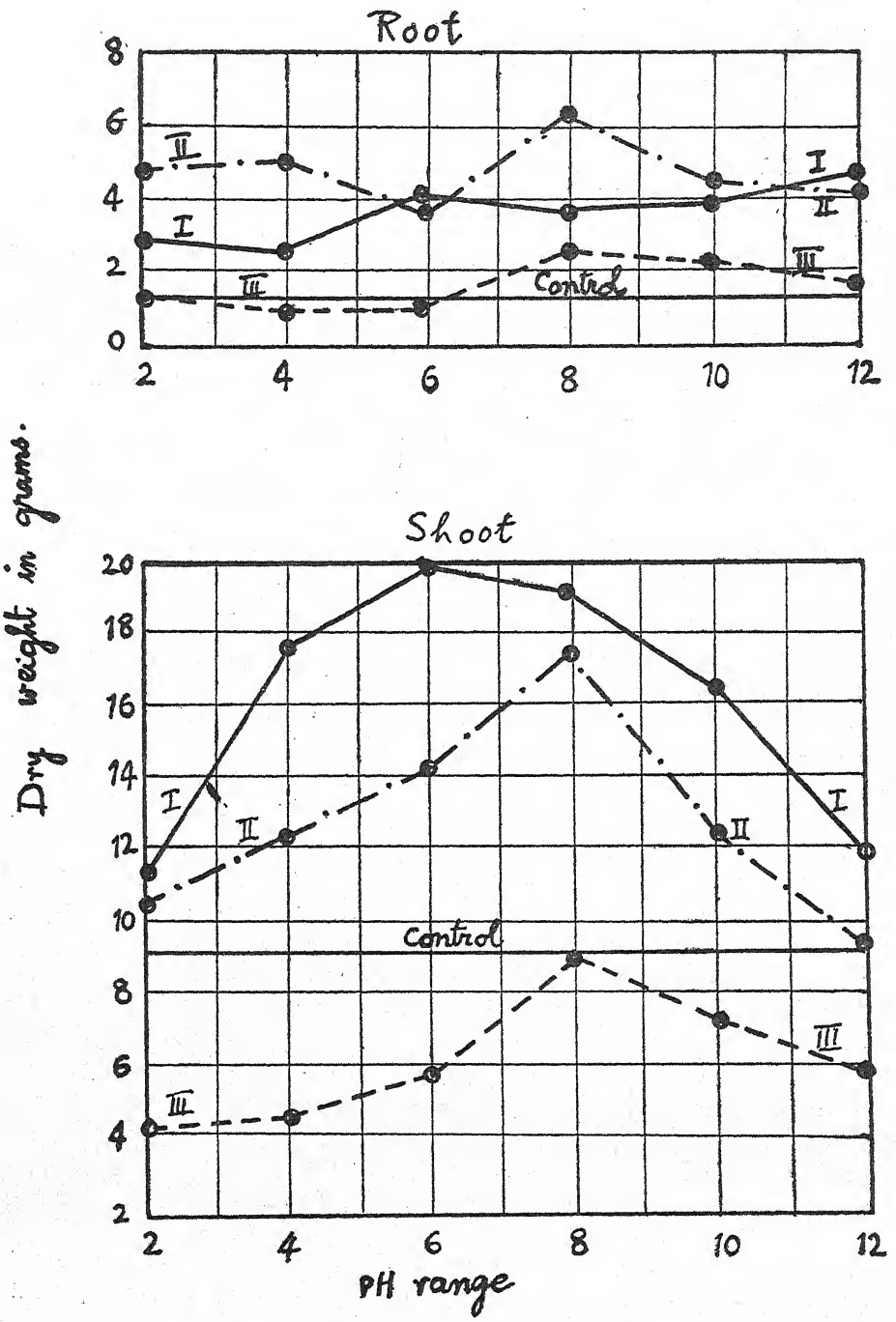


Fig. 4. Dry matter output of the shoot and root obtained from differently treated setts.

I 24 hours. II 48 hours. III 96 hours.

It is interesting to note that maximum dry matter production in all the three periods is continued to pH 8, (48 and 96 hours) and pH 6, (24 hours)—the hydrogen-ion concentrations which were found to give a better germination energy.

Buds.—The dry matter output of buds is found to decrease with the increase in the period of treatment. Thus all the setts treated for ninety-six hours give the lowest dry matter which is even lower than the control. In the case of the buds also the readings start from a low value on the acidic side, attain a maximum at pH 8 in forty-eight and ninety-six hours and at pH 6 in twenty-four hours and gradually fall down towards the alkaline side. The increase in dry matter over the control due to stimulation is significant. At pH 6 of twenty-four hours which gives the best growth of all the treated setts the dry matter output is $19 \cdot 82 \pm 0 \cdot 231$ grm., while that of the control is only $9 \cdot 24 \pm 0 \cdot 104$ grm. The increase over the control is 114·8 per cent.

Discussion

The relation of germination vigour to the production of dry matter

A higher dry matter output is found to be associated in majority of the cases with a higher germination energy. For example the setts which are treated for a period of twenty-four hours give a lower dry matter output of roots than the setts treated for forty-eight hours. As to germination energy, the former gives a maximum in majority of cases four days later than the forty-eight hour treatment. The pitch of the maximum is much lower in the case of twenty-four hour treated setts than in the forty-eight hour treated ones to which reference has already been made in the previous pages. Ninety-six hour treated setts give the lowest dry matter output and a poor germination.

The effect of germination energy on dry matter output is found to be evident even when the setts treated at various pH values are compared among themselves. For example pH 8 in forty-eight hour period possesses the best germination energy as revealed by a value as high as 62·5 per cent attained two days after germination. In the case of twenty-four hours also the best germination energy is noted in pH 6, 8, 10 and 12 all of which give nearly equal dry matter production. In the case of ninety-six hours where the majority of the curves are of flat nature a definite maximum is shown at pH 8 which also gives the highest dry weight output.

The same appears to be true to an equal extent of bud germination. As regards the dry matter output in this case, twenty-four hour period in all its pH ranges is found to have better performance than forty-eight hour which, in its turn, is better than the ninety-six hour. As far as germination is concerned excepting pH 8, in all the cases the twenty-four hour comes prior to forty-eight

hour higher duration treatment. In ninety-six hour period the maxima are invariably late when compared with shorter periods and also the starting of germination is nearly a week behind as already mentioned. Thus these facts seem to indicate that germination energy and subsequent growth, as represented by dry weight, go hand in hand.

Attention may be drawn to the observations made by Tincker [1925] in this connection. From a synopsis of the experiments described by the author it appears that a definite correlation does exist between the vigour of germination and the rate of subsequent growth in the case of oats. Such relationship has also been demonstrated by Kondo for rice [1923] in conformity with our own results discussed above.

Increment in the dry matter output due to treatment

As a result of pre-soaking treatment there is an apparent increase of 114·8 per cent in dry matter of the shoot over the control, and this occurs in the case of setts treated in pH 6 in twenty-four hour period of soaking which has shown the best growth among all the other treatments. The difference has been found to be statistically significant. This result is highly encouraging and if this can be maintained under field conditions without any appreciable decrease in the sucrose content and other desirable qualities, it will constitute a substantial advance in the sugarcane industry.

Similar increased growth due to chemical treatment has been observed by many other workers. Gleisberg [1924] noticed that the vegetative growth of radish was increased up to 364 per cent when the seeds were treated in fifteen per cent magnesium chloride and magnesium sulphate. He also noted a doubling of yield in seeds treated with distilled water. Extensive work on the effect of chemicals upon hastening germination and increasing the yield has been carried out by Denny [1926 ; 1, 2] who found best results from treatments with vapour of ethylene chlorhydrin and solutions of sodium and potassium thiocyanate. The treated tubers showed cent per cent germination and in a period of two months the plants were two feet high with a formation of tubers, while the control ones did not appear above the ground. On the other hand Smith [1930] working with wheat came to the conclusion that none of the treatments used by this author proved to be highly stimulative to growth. The failure in getting an increased growth is most probably due to the fact that the author confined himself to a single treatment and a single concentration with each of the chemicals. The works of Denny [1926 ; 1, 2] and his co-workers have clearly indicated that there are particular optimal periods and concentrations of treatment for the different crops worked upon. The optimum concentration has also been found to vary in the case of different varieties of the same crop.

Relation of sucrose and reducing sugars present in the mother sett to growth

An interesting point in the observation so far recorded is that the maximum growth of buds and sett roots generally occurs in setts which have been treated for the various periods in solutions the reactions of which are maintained slightly above or below neutrality. In order to understand whether the conversion of sucrose to glucose has got anything to do with increased growth or not, it is necessary to go back to those data which have already been referred to (Fig. 3). A study of the glucose and sucrose readings seem to indicate that wherever there is higher growth, both reducing sugars and sucrose values are relatively low. For example the sucrose values of twenty-four hours during all the three periods of analysis are low when compared to forty-eight hours. Forty-eight hour in its turn shows readings lower than those of ninety-six hours where minimum growth occurs. When an inter-comparison of setts at various pH values for the same period is made, the minimum sucrose is found to occur in setts treated with pH 8 where the growth is also the highest. The low sucrose content in case of setts where high growth prevails can be explained on the basis that at such an early stage when the offspring are not yet established independently, it is the conversion of sucrose which supplies the respiring and tissue-forming materials. Hence increased growth requires a greater amount of these growth materials and an equal amount of drain on the reserve sucrose material. This naturally seems to be the reason for the low sucrose value obtained in such vigorously growing canes. It is interesting to note that the value of glucose is relatively low where a consumption of sucrose exists. This seems to be quite natural as the reducing sugar value, that is obtained during any period of analysis, is essentially the difference between the amount of it formed from sucrose and the amount used up. High rate of growth leads to high consumption of these reducing sugars and most probably for this reason the balance is low.

The storage material being mostly sucrose in sugarcane it may be expected that a greater conversion of sucrose to reducing sugars which forms the suitable substrate for the germinating buds and sett roots should occur in the setts which are soaked in solutions of more acidic nature and that they should give earlier and better growth as compared to control, but on the contrary the best growth is not seen in such treatments.

In order to understand whether the sucrose conversion is brought about by some change in the reaction of the juice of the mother sett, a reference is made to a record of the hydrogen-ion concentration of the differently-treated setts. These records are taken only for the first period of analysis by the quinhydrone electrode. A perusal of Table I clearly reveals that the hydrogen-ion concentration varies from 5·412 to 5·583 and that there is no regularity as to their order and arrangement in relation to high sucrose conversion.

TABLE I

pH value of the sap extracted from the mother setts at the sett-root germination stage

Setts treated at pH	24 hours	48 hours	96 hours
2	5·471	5·518	5·496
4	5·498	5·480	5·412
6	5·467	5·583	5·483
8	5·516	5·502	5·559
10	5·441	5·498	5·442
12	5·492	5·529	5·537
	Control	5·465	

In pH 6 and 8 of twenty-four hours, where growth and sucrose conversion are at the maximum, the pH values are 5·467 and 5·516, but these seem to be almost similar to the pH value of the setts treated at pH 2 where the growth and sucrose conversions are at minimum. The same seems to be the case with forty-eight hour and ninety-six hour periods also. It appears that the reaction of the sap has not much to do with the increased growth and sucrose conversion.

The exact mechanism by which the enhancement of growth occurs still awaits investigation and it may be found in other aspects of the metabolism of the stimulated individuals.

SUMMARY AND CONCLUSIONS

The present investigation is an attempt to study the effect of pre-soaking of sugarcane in solutions of different hydrogen-ion concentrations upon germination and growth of seedlings. The following observations are recorded :—(*i*) germination energy of sett roots and buds, (*ii*) march of glucose and sucrose contents in the mother setts, (*iii*) the hydrogen-ion concentration of the juice and (*iv*) the dry-matter output of both sett roots and buds when harvested two and a half months after sowing.

The differently-treated setts are found to vary both as regards germination energy and dry-matter output. There seems to exist a positive correlation between the two. The maximum growth is found to occur at pH 6-8. The best result is obtained for the sett-root growth in forty-eight hour treatment, while that

for the growth of shoot is exhibited by the setts treated for a period of twenty-four hours. Thus the sett roots and buds are found to be stimulated differently by the treatment. All the treatments, except that of ninety-six hours, have shown an increase in the production of dry matter over the control. In pH 6 of twenty-four hours duration the increase is up to 114·8 per cent.

The sugar-analysis records indicate a greater conversion of sucrose into glucose and also a greater consumption of the latter under treatments which give the best growth.

Contrary to expectation this greater conversion occurs near about the neutral side of the reaction. The records of the hydrogen-ion concentration indicate that the difference in the behaviour as to the sucrose conversion in the differently-treated setts cannot be explained on this basis. No correlation is found to exist between the two. It is suggested that the exact cause of stimulation should be searched in other aspects of the metabolism of the treated setts.

The present investigation opens up an apparently new field of enquiry for sugarcane and further investigation may be in a position to furnish some treatment which may be of direct benefit to the cultivator in getting an increased yield.

REFERENCES

Denny, F. F. (1926,1). *Amer. J. Bot.* **13**, 118-125.

———— (1926,2). *Ibid* **13**, 386-396.

Gleisberg, W. (1924). *Deut. obst. u. gemusebau Ztg. Woch. Ausg.* **70**, 4, p. 28 (*Exp. Stn. Rec.* **50** No. 7,643, 1924).

Harrington, G. T. (1923). *J. Agric. Res.* **13**, 2, 79-100.

Jones, M. G. and Tincker, M. A. H. (1926). *Ann. App. Biol.* **13**, 4, 535-559.

Kondo, M. (1923). *O'Hara. Inst. Bull.* **Bd. II**, Heft 3, 291.

Smith, D. C. and Bressman, E. N. (1930). *J. Agric. Res.* **40**, 25-36.

Tincker, M. A. H. (1925). *Ann. App. Biol.* **12**, 440-469.

Venkatraman, T. S. amd Thomas, R. (1924). *Agrc. J. Ind.* **14**, 509-514.

Willaman, J. J. and Davison, F. R. (1924). *J. Agric. Res.* **28**, 5.

A NOTE ON PHOTO-PERIODISM IN SESAMUM

BY

D. RHIND, I. A. S.,

Economic Botanist, Burma

(Received for publication on 16th April 1935)

(With Plates XXXI-XXXIII)

INTRODUCTION

The Burmese have recognised for a long time that there are two distinct classes of sesamum which they distinguish by the names *Hnanyin* and *Hnangyi*, indicating that the former class are for growing during the monsoon and the latter class during the cold weather. In Burma it has been customary to designate these two classes "early" and "late" in English irrespective of what the life-period may be [Rhind and Ba Thein, 1932]. The "early" class are usually sown from April to the end of June and are harvested in August or September though they can be sown as late as October. If, however, they are sown late, the growth is curtailed and yield suffers. The "lates" are sown from September to October and harvested in December or January. If they are sown in the "early" season, growth is rank and coarse but flowering is either entirely suppressed or the flowers are abnormal and fail to set fruits.

Rhind and Ba Thein [1932] have shown that the "late" character appears to be a simple dominant over "early".

EFFECT OF SOWING DATE ON "LATE" SESAMU

In 1934 two types of "late" sesamum were sown in field plots at Mandalay (Lat. 22°N.) at weekly intervals commencing on 15th June. The types were *Boktaung L 31-33*, a long-lived type, fully branched, with four-locular, solitary capsules and brown seed, and *Thadunbyu L 31-12*, a short-lived, unbranched type with four-locular, solitary capsules and white seed. The results are summarised in Tables I and II.

TABLE I

Effect of sowing Boktaung L 31-33 *on various dates*

Sowing date	Date of 1st gland	Date of 1st flower	Fruit	Sepaloid plants per cent	Total No. of plants	Hours of day light at sowing date	Notes
I 15th June 1934	12th Sep. 1934	*Nil*	*Nil*	32	147	13·28	See (1) below.
II 22nd June 1934	,,	27th Sept. 1934	Few	30	131	13·28	Do.
III 29th June 1934	,,	27th Sep. 1934	Few	24	166	13·28	See (2) below.
IV 6th July 1934	,,	12th Oct. 1934	Many	24	154	13·26	See (3) below.
V 13th July 1934	27th Sep. 1934	,,	Many	16	233	13·26	Do.
VI 20th July 1934	...	,,	Many	10	282	13·18	
VII 27th July 1934	...	,,	Many	4	192	13·14	See (4) below.
VIII 3rd Aug. 1934	6th Oct. 1934	,,	Many	1·5	269	13·08	See (5) below.
IX 10th Aug. 1934	,,	...	Many	9·5	238	13·02	See (6) below.
X 17th Aug. 1934	,,	12th Oct. 1934	Many	1·1	260	12·56	See (7) below.
XI 24th Aug. 1934	12th Oct. 1934	...	Many	0·0	...	12·48	Do.
XII 31st Aug. 1934	,,	...	Many	0·0	...	12·40	Do.
XIII 7th Sep. 1934	...	...	Many	0·0	...	12·34	Do.
XIV 14th Sep. 1934	...	...	Many	0·0	...	12·26	Do.

(1) The general appearance of this plot on 27th September (104 days from sowing) was tall, rank plants, with little branching below two feet and much branching in the uppermost foot (top-branching, Plate XXXI, fig. 1). The upper small branches were bearing flowers but setting very few capsules. Plants which showed sepaloid growth a month ago, now bearing apparently normal flowers.

2) These plants also showed top-branching but less pronounced than I and II. There was some sepaloidy but by 27th September (91 days from sowing) a few apparently normal flowers were produced though they failed to set fruits. Some plants ultimately produced normal growth after having shown the sepaloid condition in the early stages.

(3) Typical growth of the "late" class when grown in the "early" season, with profuse, rank growth and top-branching. After having shown the sepaloid condition, flowering and fruiting occurred by the middle of October.

(4) By 12th October plants were flowering freely but set very few fruits. The sepaloid condition occurred in only eight plants out of 192 and these were not badly affected. Ultimately fruiting became almost normal on the upper parts of the plants.

(5) Branching was mostly at the top in the early part of the life-period. Flowering and fruiting finally became about normal.

(6) The growth was nearly normal, only slightly more rank than when this class is sown in its proper season. The plants affected with sepaloidy were all in one group in the plot, 23 out of 238, and the higher percentage in this case cannot be explained.

(7) Normal growth.

FIG. 1. Late variety *Boktaung L31-33*, sown 22nd August, photographed 28th September, showing top-branching. The plant on the right is commencing to bear normal flowers at the top.

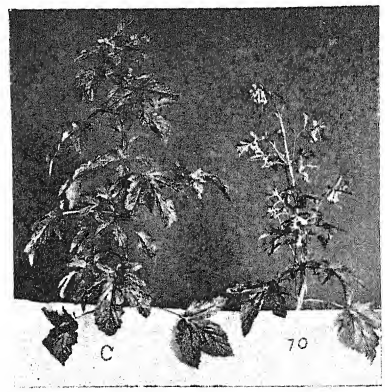


FIG. 3. Late variety *Boktaung L31-33*, sown 6th July, photographed 12th September, control (left) and after 70 hours extra darkness.

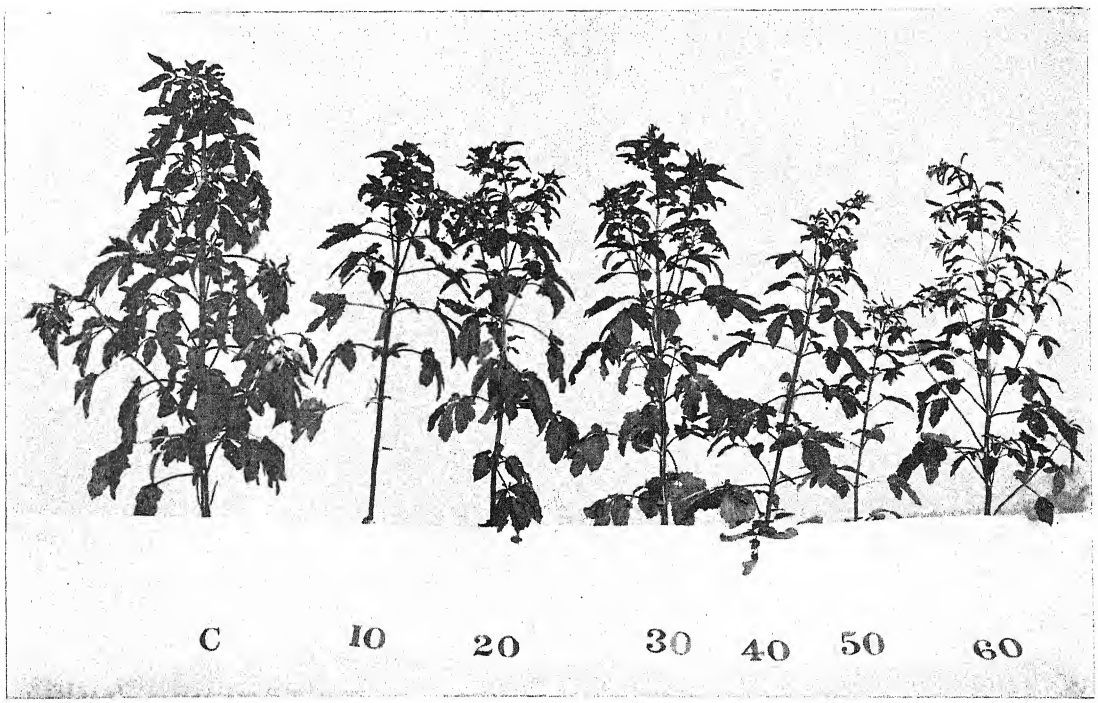


FIG. 2. Late variety *Boktaung L31-33*, sown 15th June, photographed 11th September. Given 10 to 60 hours extra darkness.

TABLE II

Effect of sowing Thadunbyu L 31-12 *on various dates*

Sowing date	Date of 1st flower	Fruiting	Sepaloid plants per cent	Total No. of plants	Hours of day light at sowing	Notes
I 15th June 1934	12th Sept. 1934	Few	100	...	13·28	See (1) below.
II 22nd June 1934	.....	*Nil*	100	...	13·28	
III 29th June 1934	12th Sept. 1934	*Nil*	99	...	13·28	
IV 6th July 1934	Ditto	Few	98	...	13·26	
V 13th July 1934	Ditto	Few	75	127	13·26	See (2) below.
VI 20th July 1934	Ditto	Few	67	127	13·18	Do.
VII 27th July 1934	14th Sept. 1934	Few	50	80	13·14	
VIII 3rd Aug. 1934	14th Sept. 1934	Few	41·8	189	13·08	
IX 10th Aug. 1934	27th Sept. 1934	Few	41·2	119	13·02	
X 17th Aug. 1934	27th Sept. 1934	Many	35·4	133	12·56	
XI 24th Aug. 1934	27th Sept. 1934	Many	11·1	224	12·48	
XII 31st Aug. 1934	6th Oct. 1934	Many	2·8	211	12·40	
XIII 7th Sept. 1934	.....	Many	22·9	227	12·34	See (3) below.
XIV 14th Sept. 1934	.....	Many	21·2	250	12·26	

(1) There is some doubt whether this line is pure. There were scarcely any fruits matured and all plants showed the sepaloid condition.

(2) Practically all plants were sepaloid in the early stages but many recovered and formed a few capsules.

(3) The increase in percentage of sepaloidy in the last two sowings is not understood.

In the case of *Boktaung* the sowings prior to 3rd August developed abnormally with few flowers or axillary glands until late in the season when sparse flowering and fruiting with much phyllomania (sepaloidy) occurred. Sowings after the 3rd August developed normally with good fruiting and little or no sepaloidy. The demarkation between the heavy vegetative growth and normal growth was, of course, not sharp, but 3rd August marked the approximate boundary between normal and abnormal growth. Plants which produced sepaloid growth in the early stages frequently produced normal flowers and fruits later in the season. It is noteworthy that with the exception of the sowing on 10th August there was a continual decline in the percentage of sepaloid plants (recovered plants were recorded as sepaloids). Further, in the sowings of the short-lived variety *Thadunbyu* on 7th and 14th of September there was also a rise in the sepaloid percentage. There may have been some climatic cause, but none could definitely be ascertained.

In the case of *Thadunbyu* there was some indication of impurity and the distinction between the heavy, rank growth of " lates " sown too early and normal growth was not always clear. But it is clear that early sowings lead to excessive vegetative growth and much sepaloidy. Sowings from 10th August onwards gave a large number of normal plants.

EFFECT OF REDUCING THE HOURS OF DAY LIGHT

On the 9th July certain plants selected at random growing in the plots sown on 15th June were covered with large flower-pots from 3-30 P.M. to 6-30 A.M., thus reducing the normal period of daylight by about four hours. The treatment was discontinued on 19th July when a total of forty hours extra darkness had been given. The following effects were noticed :—

19th July.—Varieties *Boktaung* and *Thadunbyu*. Covered plants three inches taller than the uncovered.

26th July.—Boktaung: Covered plants three to five inches taller than the uncovered. Bearing axillary glands and flower buds up to 0·25 in. across. Uncovered had no glands or buds.

Thadunbyu: Covered plants up to six inches taller. Axillary glands and buds well formed, the latter up to $\frac{3}{4}$ in. long.

29th July.—Thadunbyu: Covered plants in flower. Flowers appear normal. Uncovered not showing buds or axillary glands.

30th July.—Boktaung: One covered plant with one flower open. Uncovered show neither buds nor glands.

10th August.—Thadunbyu: Covered plants flowering and setting fruits. Flowering slowing up as the effect of the treatment appears to be wearing off.

Boktaung: Covered plants flowering but only one capsule set. No buds on untreated plants of either variety.

17th August.—Thadunbyu: Covered plants flowering and fruiting. Some plants sepaloid. Upper leaves lanceolate, entire. Some untreated plants flowering ; no fruits set. A few upper leaves broad, lanceolate but mostly tripartite. Some sepaloid.

Boktaung: Covered plants flowering. One capsule set. Upper leaves lanceolate, entire. None sepaloid. Untreated show no buds or glands. Upper leaves tripartite, serrate.

From these observations it is seen that the small amount of extra darkness given, amounting to about four hours per day, was sufficient in ten days to induce flowering and to bring about the formation of the narrow lanceolate type of leaf usually found on the upper parts of flowering sesamum plants. The treatment was not continued beyond the ten days and the plants later reverted to the rank vegetative type of growth.

In a second experiment on the *Boktaung* variety sown on 6th July varying periods of extra darkness from ten to seventy hours in all were given. Specimens of these treated plants and of the control are shown in Plate XXXI,

figs. 2 and 3. The treatments were commenced on 2nd August and on the 11th September the following notes were made :—

Control.—Very coarse, rank vegetative growth. Leaves large. Stem thick, branched. Minute glands and flower buds discernible on some plants.

Ten hours.—Tall, rank. Upper leaves ovate or ovate-lanceolate, scarcely entire. Glands and very small flower buds formed.

Twenty hours.—Rank growth, tall, branched, stem thick, leaves large, upper leaves broad-lanceolate. Glands and small flower buds formed at apex.

Thirty hours.—Same as twenty hours.

Forty hours.—Plants branched ; growth rather coarse, stem thick, foliage coarse. Glands and flower buds formed at apex but not opened.

Fifty, sixty, seventy hours.—Plants shorter than controls. Branched. Flowering. Some capsules about half developed. Upper leaves lanceolate. Plants have general appearance of growing normally.

The increasing effect of the longer periods of darkness is well shown. The experiment was repeated on the same variety sown on 27th July with the same results.

It is clear that when the "late" class sesamums are grown under long days, they tend to produce excessive vegetative growth while reproduction is suppressed or rendered abnormal. If the days are artificially shortened, flowering is induced and also the other changes, such as gland production and alteration in the shape of the upper leaves.

Effect of lengthening the light-period on "late" sesamum grown under short days

In order to test the above results further, a series of pots was sown on 22nd September with the following four varieties of "late" sesamums and four varieties of "early" sesamums :—

	Pot Nos.
Lates	
Thadunbyu L 31-12	1-19
Chauklaknan L 31-18	20-38
Boktaung L 31-33	39-59
Hnanni-gyi L 31-50	60-78
Earlies	
Shithmyaung E 25-187	79-89
Hnan-net E 27-47	90-102
Thadunbyu E 26-136	103-112
Palestine Northern Plain E 34-72	113-120

The germination was good. There was some trouble with damping-off but one spraying with ½ per cent Bordeaux mixture stopped this. The seedlings were thinned out to two per pot. The pots were arranged in sets of four of each variety in the form of a 4 × 4 Latin square under 75-watt, gas-filled lamps calculated to give an average of 6·7 foot candles at the soil surface. Extra electric light was given from sunset for two, four and six hours to three sets of the " lates " and for four hours to one set of the " earlies ", commencing on 6th October when the plants were fourteen days old. There was a failure of the lights on 9th October due to a short circuit but at no other time. The longest extra dose of light, six hours, is only approximate as the switching off of this series was done by the night watchman, the lamp being on the local street-lighting circuit. It is nevertheless approximately correct. The extra illumination affected both growth and reproduction, which will be dealt with separately.

Growth (*' lates '*).—The effect of extra illumination is to reduce growth in height, the effect increasing as the extra period of electric light is increased (Plates XXXII and XXXIII). The plants under four and six hours extra light did not produce the narrow lanceolate upper leaves but only tri-lobed, serrate leaves. They were paler in colour than the controls.

Measurements of heights were made weekly but only the final measurement made on 12th November need be reproduced here. The average heights then were :—

Control	43·57 cm.
Two hours	28·66 ,,
Four ,, ...	24·78 ,,
Six ,, ...	22·38 ,,

The differences between each treatment and the control are all significant ($P < 0·01$) though inter-treatment differences are not significant. Nevertheless, when the plants were arranged in order of increasing periods of illumination there was, with few exceptions, a steady decrease in height as the time of illumination increased. Inter-varietal differences, though considerable in some cases in the two-hour treatment, were not reliable.

Reproduction (*' lates '*).—The controls commenced to flower on the 23rd October in the case of *Thadunbyu* and *Chauklahnan* (short-lived) varieties and on 5th November in the case of *Boktaung* and *Hnanni-gyi* (long-lived) varieties. Flowering and fruiting proceeded normally ; there was no sepaloidy and the plants were ripe about the 10th December.

Of the treated plants only one variety, *Thadunbyu L 31-12*, flowered. The flowering was irregular and only eleven out of the total twenty-four treated plants flowered. These set four capsules. The flowers appeared on various dates between the 25th October and 10th December. The other three varieties

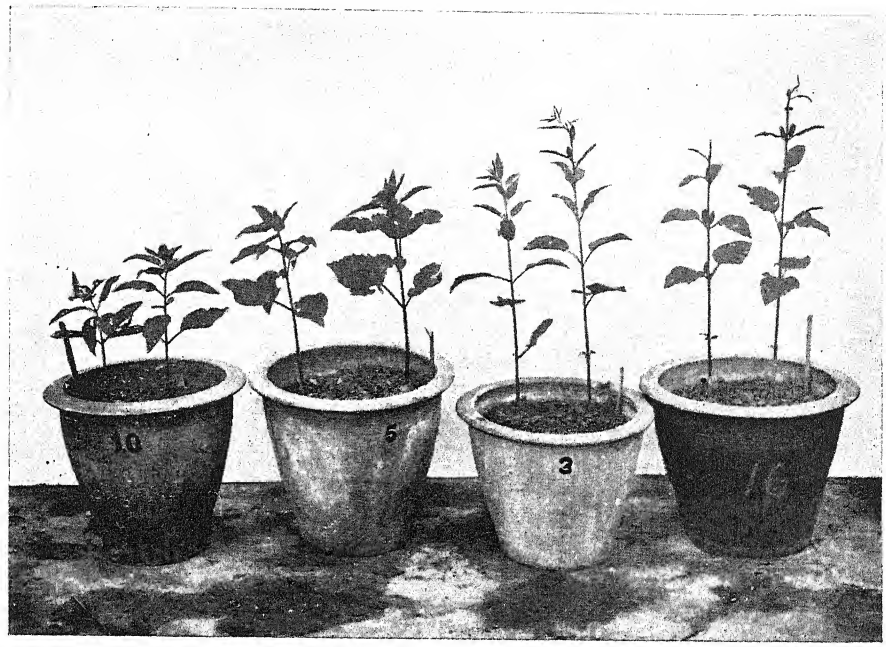


FIG. 1. Late variety *Thadunbyu L31-12.* From right : control, 2 hours, 4 hours and 6 hours extra light.

FIG. 2. Late variety *Chauklahnan L31-18.* From right : control, 2 hours, 4 hours and 6 hours extra light.

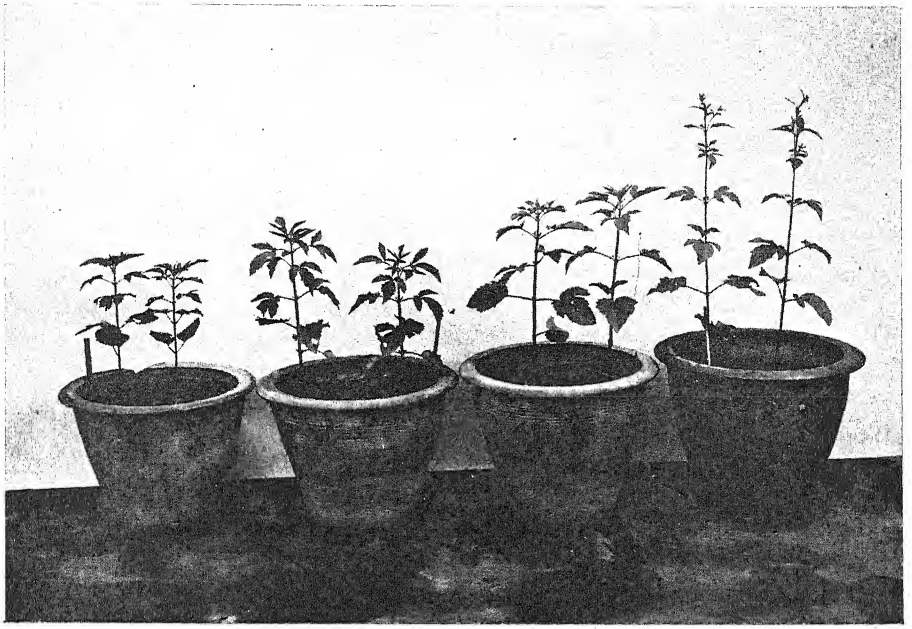


FIG. 1. Late variety *Boktaung L31-33*. From right : control, 2 hours, 4 hours, and 6 hours extra light.

FIG. 2. Late variety *Hnangyi L31-50*. From right : control, 2 hours, 4 hours and 6 hours extra light.

did not produce a single flower and the plants finally died towards the end of December. There were glands and small flower buds developed in a few cases on the two-hour and four-hour sets but they failed to expand.

The effect of artificial long days, even with the low intensity of light employed, was very marked and clearly shows that the *Hnangyi* ("late") class are only capable of normal growth and reproduction under relatively short days. At the latitude of Mandalay the mean time of exposure to light (day light + electric light) for the period from 24th September (date of germination) to the 30th November was

	Hours	Minutes
Controls	11	22
Two hours	13	1
Four hours	14	40
Six hours	16	18

(The extra illumination was given for a total of fifty-six days from 6th October).

The additional one hour 39 minutes given to the two-hour series on an average over the fifty-six days from 6th October to 30th November had a pronounced effect, completely suppressing flowering except in one variety and even in that case greatly curtailing it and permitting only a few fruits to set. The average length of day during the normal "early" season from 15th June to 31st August is thirteen hours eleven minutes and under a day of this length the behaviour of "lates" is quite in accordance with that of short-day plants described by other workers, *e.g.*, Garner and Allard [1920], Tinckler [1925; 1928], Adams [1923] and McClelland [1928]. It may be mentioned that Pusa Types 5, 17, 25, 26, 27, 28, 29 and 30 have been found to be short-day varieties when grown in Burma.

Growth ('*earlies*').—The mean heights of the plants receiving four hours extra electric light was 43·15 cm. and the controls 44·35 cm., the difference being insignificant. Both series grew and fruited normally and produced the usual lanceolate top leaves.

Reproduction ('*earlies*').—The dates of opening of the first flower were considerably delayed by the extra period of illumination. All plants of the same variety did not flower on the same day and the dates given below are those for the majority of the plants of each variety. The Palestine Northern Plains variety in particular was irregular in its flowering :—

	Control	Treated
Shithmyaung E 25-187	18th October	22nd October
Hnan-net E 27-47	22nd ,,	10th November
Thadunbyu E 26-136	20th ,,	31st October
Palestine N. P. E 34-72.	19th-27th Oct.	23rd Oct. to 3rd November

The extra llumination has delayed the opening of the first flower from four to nineteen days, the delay being greatest in the case of the longest lived variety, *Hnan-net*. There was no sepaloidy and the plants fruited normally.

Summary

The so-called "late" sesamums (Burmese *Hnangyi*) are typical short-day plants which only grow and reproduce normally under days of about twelve hours or less. An extension of the light period to thirteen hours causes abnormal growth and suppression of flowering.

The "early" sesamums (Burmese *Hnanyin*) are not restricted to any particular day length and are able to flower and fruit under either long or short days though growth and yield are best under long days.

REFERENCES

Adams, J. (1923). *Ann. Bot.* **37**, 75.

Garner, W. W. and Allard, H. A. (1920). *J. Agric. Res.* **18**, 553.

McClelland, T. B. (1928). *J. Agric. Res.* **37**, 603.

Rhind, D. and Ba Thein (1932). *Ind. J. Agric. Sci.* **3**, 478.

Tinckler, M. A. H. (1925). *Ann. Bot.* **39**, 721.

——————— (1928). *Ann. Bot.* **42**, 101.

LIFE-HISTORIES OF SOME INDIAN THYRIDIDAE (LEPIDOPTERA)

BY

B. B. BOSE, B.Sc. (ALLD.),

Assistant to the Imperial Entomologist, Imperial Institute of Agricultural Research, Pusa

(Received for publication on 18th May 1935)

(With Plates XXXIV-XXXVI)

INTRODUCTION

The family Thyrididae is closely allied to the family Pyralidae, so much so, that even trained entomologists are likely to be misled about the exact identity of the larvae and sometimes of the adults of these two families. The moths of the Thyrididae are also likely to be mistaken for those of the Geometridae. The "Pyralid larva forming galls on the twigs of *Phyllanthus emblica*," referred to by Misra [1919] is most probably the Thyridid larva *Betousa stylophora* Swinh., because so far this is the only species which is known to form galls on this plant at Pusa.

The working out of the life-histories of the species belonging to this family involves special effort and that is probably the reason that the detailed life-history of not a single species was known up till 1925, in which year the writer was successful, after repeated failures, in rearing the adults of one species, *viz.*, *Betousa stylophora* Swinh. and later on, of the other species, *viz.*, *Rhodoneura loceusalis* Wlk. The observations on the life-histories of these two insects, together with brief notes on other species of this family taken from the records in the office of the Imperial Entomologist, are embodied in this paper. A brief account of one of these species was given by Fletcher [1931] at a meeting of the Entomological Society of London in 1930.

As various reasons and other pressing duties have prevented the writer from completing a detailed study of these two species, it has been thought desirable to publish such information as has been collected.

I.—*Betousa stylophora* Swinh. (Plates XXXIV and XXXV; Figs. 1, 2, 3, 4, and 5, 6, 7)

Swinhoe [1895] described this species as *Rhodoneura stylophora*. Hampson [1896] first described it as *Hypolamprus stylophorus* but in 1914 included it under *Betousa* Wlk. on account of the priority of the latter genus.

Phyllanthus emblica (Euphorbiaceae) on which this species forms galls, is of some economic importance in India, its fruit being used as pickles, jam, medicine, etc. Fresh galls are generally formed in the summer months, June to August. They often attain maximum size by the beginning of winter, when they are about 23·1 mm. in length and 10·3 mm. in width. The galls when young, are proportionately much longer than broad (Plate XXXIV, figs. 1, 2). Often the galls are distinct from one another, but sometimes two galls are contiguous, having a narrow constriction in between them.

Some empty galls out of which the moths had emerged were found to be occupied by a species of small, black ants along with its young ones. Similarly once a spider and its eight round creamy-coloured eggs were found in a gall.

The writer has not seen the eggs of *Betousa stylophora* Swinh. but presumably they are laid in early summer. The caterpillars bore their way into the interior of the twigs, the bored portion swells up and in due course develops into a gall.

The caterpillar feeds inside the gall (Plate XXXIV, fig. 1) on the internal woody tissue, and passes out reddish frass through a small hole at one end which is kept covered by a mesh-work of silken threads (Plate XXXIV, fig. 2). The larva is nearly full grown about October, it passes the winter season in the larval stage within the gall and comes out in the beginning of the following summer by enlarging the entrance hole, and prepares a cocoon or pupal chamber on the leaf. A mature gall if half split with a knife, was observed to be soldered up immediately by the caterpillar inside by means of silken threads.

In the Insectary, out of some galls collected in March 1925, the caterpillars began emerging in the last week of April and continued coming out up to the end of May. A full-grown caterpillar (Plate XXXV, fig. 5) measures about 10—11 mm. in length and 2·25—2·5 mm. in breadth across the 3rd or 4th abdominal segment. It is cylindrical, tapering gently in the anterior and posterior regions. The head is darkish brown and measures 1·3 mm. across the epicrania and above the ocellar region. The epicranial suture is fine and almost colourless. The upper halves of the epicrania have shallow and closely situated pits in small patches. The ocelli are colourless. The ventral surface of the head and mouth parts are creamy-yellow, with the bases of the maxillae being black. The pro-thorax is narrower than the meso-thorax and has a glossy, lightly chitinized shield covering the whole of the dorsal surface. The three pairs of thoracic legs are glossy, and

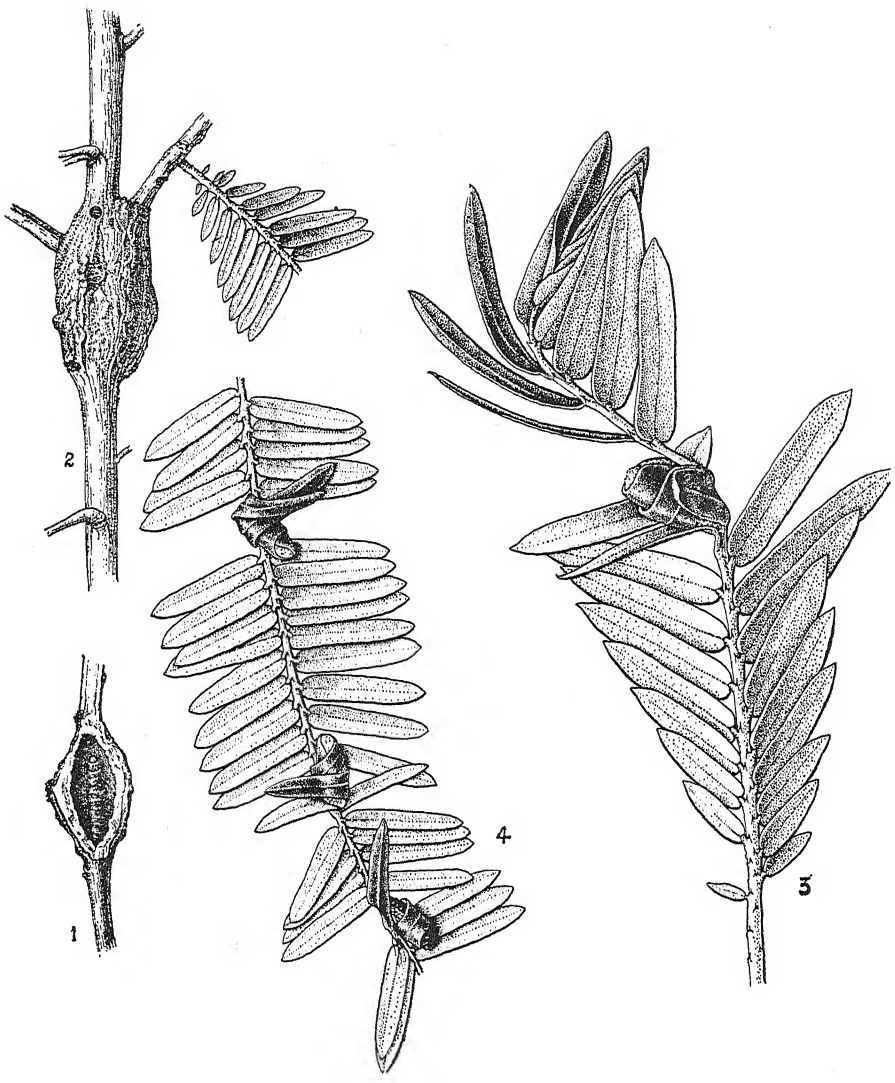


FIG. 1. Longitudinal section of gall showing the larva within (natural size).

FIG. 2. Gall showing the larval emergence hole (natural size).

FIG. 3. Leaf showing pupal chamber formed in nature (×2).

FIG. 4. Leaf showing construction of pupal chamber in captivity (natural size).

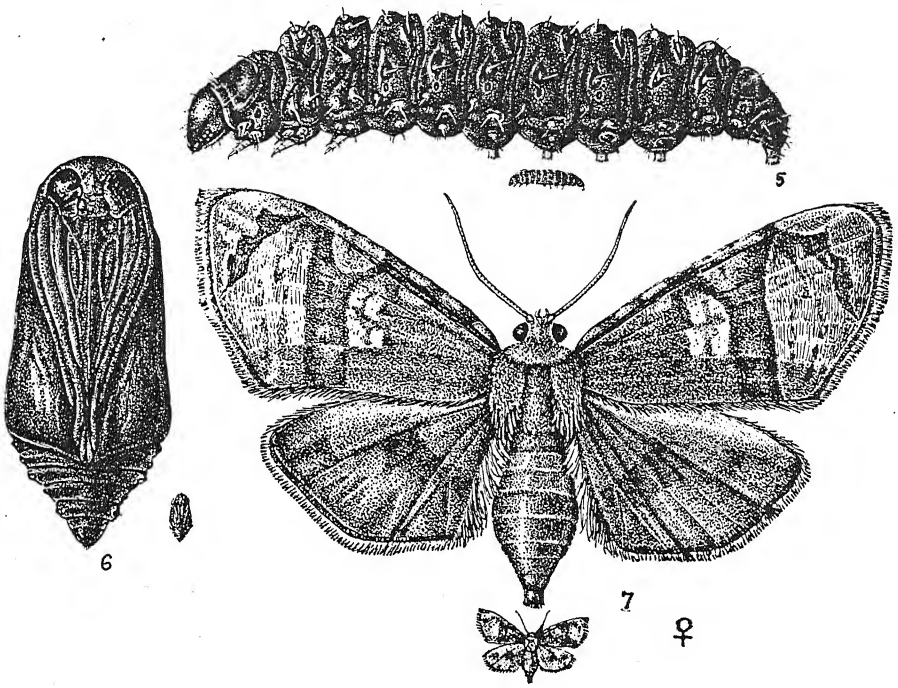


FIG. 5. Caterpillar, lateral view (× 8).

FIG. 6. Pupa, ventral view (×8).

FIG. 7. Moth (×6).

(Small figures—natural size).

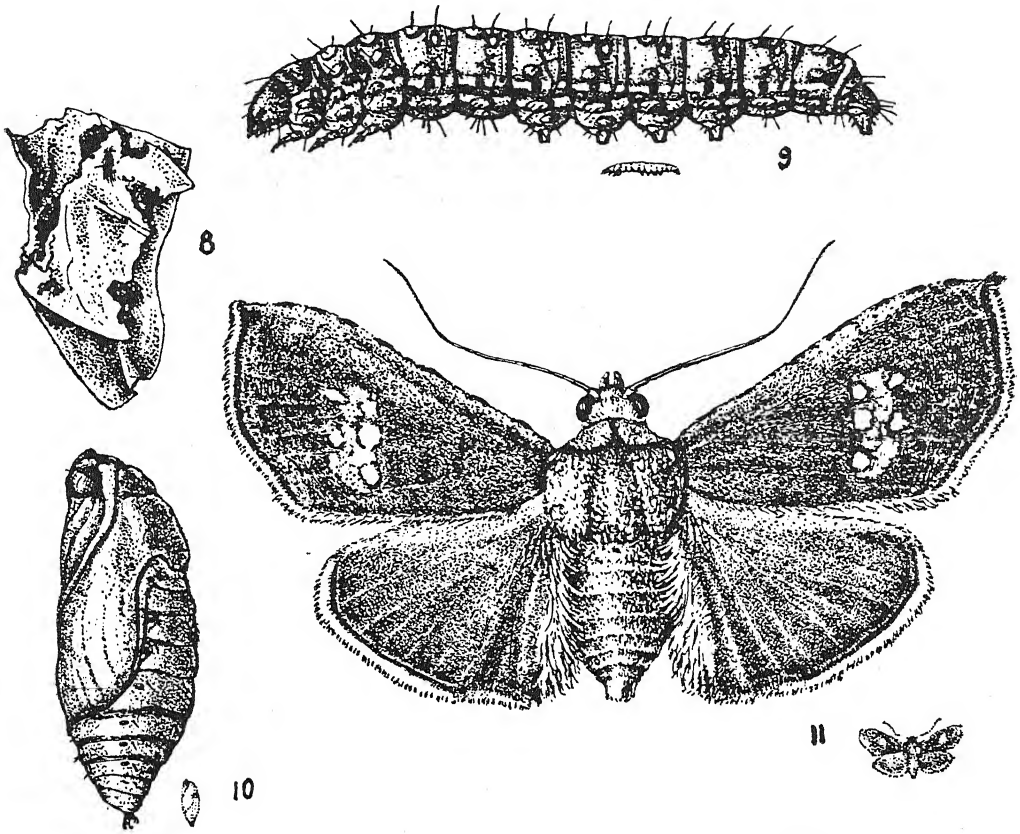


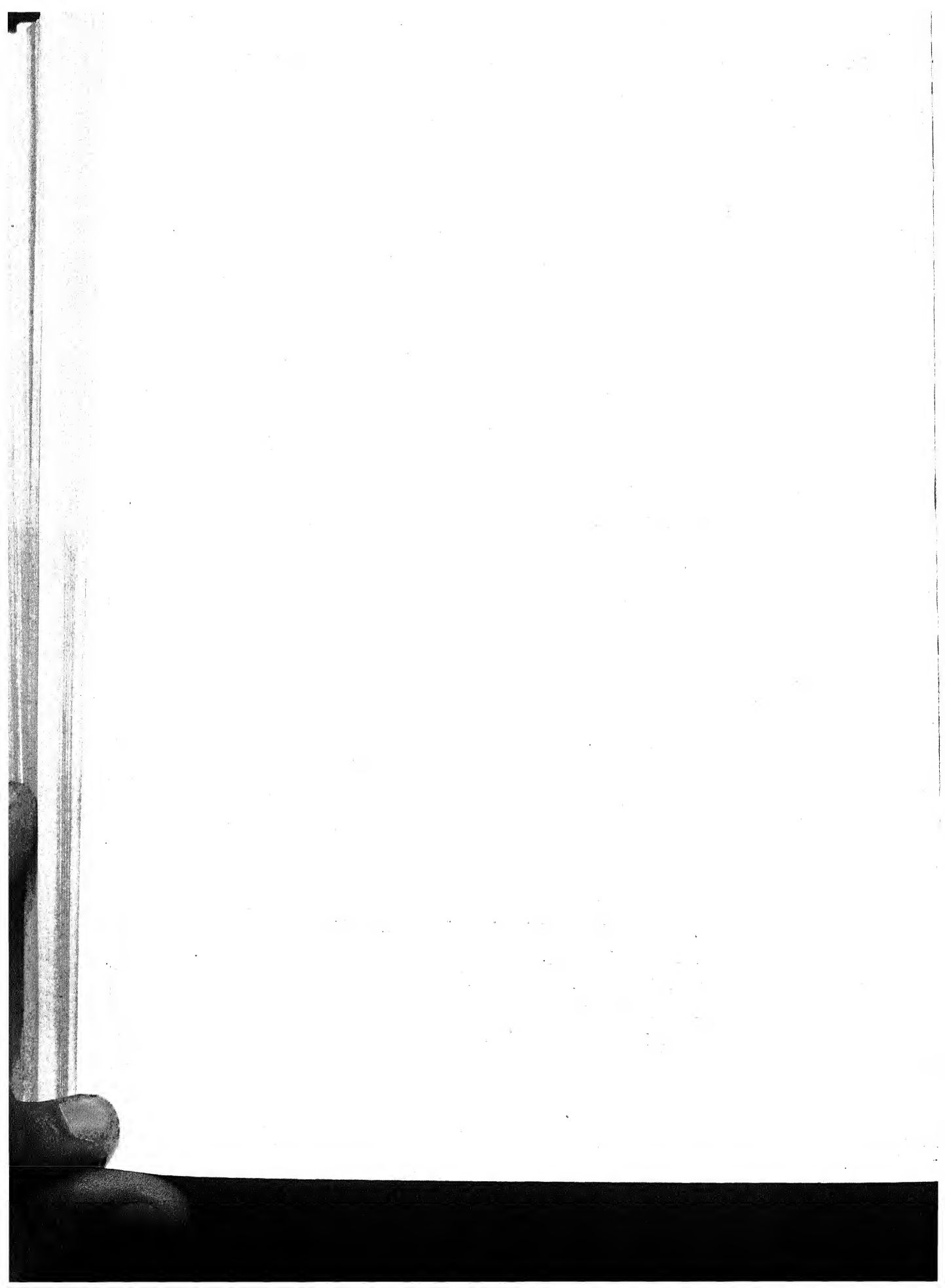
Fig. 8. Leaf-bits containing the feeding, larva within.

Fig. 9. Caterpillar (×8).

Fig. 10. Pupa (×8).

Fig. 11. Moth (×8).

(Small figures—natural size).



creamy-yellowish in colour. The tarsi and claws are brown and darker brown respectively. The colour of the rest of the thorax and abdomen is jet-black. The various body segments are distinct and have minute, fine, brownish hairs which arise singly on the dorsal surface from patches which are arranged trapezoidally on the first to eighth abdominal segments. The anterior pair of patches on each segment are nearer the mid-dorsal line and are much smaller in size than the posterior pairs which are wider apart. The tenth segment constituting the anal plate is glossy, lightly chitinized and dark sepia-brown in colour. The entire skin of the body appears to be shagreened closely. The hooklets of the prolegs are arranged in the form of a complete circle on the first four pairs, but semi-circularly in the anal claspers. The spiracles are minute, oval and black in colour and have thick rims, the first and the last pair of spiracles being larger in size than the rest.

The caterpillars form pupal chambers in the following manner (Plate XXXIV, fig. 4) :—

When full grown, the caterpillars come out of the galls and start making pupal chambers. Five leaflets are used in the preparation of each chamber. Three of these leaflets are utilized to form the base of the chamber. The basal part of these three leaflets are stuck together by means of silken threads to form the base, the remaining three-fourths are left free, sticking out like fingers. The middle portion of the chamber is formed with the other two leaflets. The leaflet of the right side of the rachis is turned into a spiral towards the left and that of the left side towards the right, the two leaflets together forming the barrel or body of the chamber. The upper end is covered by a close mesh-work of silken threads with a deposit of chewed and regurgitated leaf tissue over it. Any other chink is similarly closed up so that the chamber is well closed on all sides. The leaflets help much to give strength and safe shelter to the pupa inside and the formation of such a cocoon involves a very little secretion of silk. The caterpillars pupate within the chambers about two days after emergence from the galls. In the absence of these chambers they fail to pupate. The pupa (Plate XXXV, fig. 6) measures about 6 mm. in length and about 2·75 mm. in breadth across the fourth abdominal segment. It is beautiful red in colour. The wing cases reach the middle of the fifth abdominal segment ventrally. The visible spiracles are situated on the fourth to eighth abdominal segments; they are elongate, oval, narrow and have light brown rims. In Pusa, the pupal period lasts from eight to ten days in the month of May and June. The moth (Plate XXXV, fig. 7) emerges through the anterior end of the pupal chamber.

Distribution of the species.—In the Pusa collection, in addition to the fourteen specimens reared from caterpillars collected at Pusa, there is one moth which was collected at Kurseong. Hampson [1896] recorded the species from the Khasis hills and Ceylon.

II. *RHODONEURA LOCEUSALIS* WLK. (PLATE, XXXVI, FIGS. 8-11)

The egg stage is not known yet.

The caterpillars of *Rhodoneura loceusalis* Wlk. which feed on leaves and bore tender top shoots of *Loranthus longiflorus* have been collected at Pusa in November to April and then again in July and August. It is quite probable that they breed on this food plant throughout the year.

The caterpillars bind two tender leaves together and feed between them (Plate XXXVI, fig. 8) either by nibbling on the green tissue in small patches leaving the lower epidermis intact or feeding upon entire portions of the leaves. They also bore top-shoots and roll up the tender leaves, or feed on thick petiole and leaf under cover of meshes of silken threads overlaid with larval frass. The caterpillars shun light and prefer to feed under cover.

A full-grown caterpillar (Plate XXXVI, fig. 9) measures about 10·5 mm. in length and 2—2·25 mm. across the middle of the abdomen. It is cylindrical, tapering gradually in the posterior region. The head is shiny, yellowish-brown, bilobed, with its base slightly retracted within the pro-thorax. There is a small black marking laterally on each occiput at the base. The pro-thoracic shield is shiny, yellowish brown, concolorous with head, chitinized and has a faint thin colourless longitudinal stripe along the middle line ; the anterior thin transverse margin is also colourless. The three pairs of thoracic legs are colourless or creamy-yellowish, glossy, and their tarsi are yellowish-brownish. The colour of the rest of thorax and the abdomen is creamy-yellowish. Each body segment bears shiny, lightly chitinized, round or oval patches, each bearing one, two or three minute hairs. The five pairs of prolegs are short, cylindrical, glossy and colourless. The first four pairs have complete rings of brown hooklets, whereas the fifth or the anal claspers have the hooklets arranged in a semi-circular fashion.

All the nine pairs of spiracles are prominent, black, roundish-ovalish in shape and have thick black rims. The first and the last pair are larger in size than the rest. The dorsal surface of the anal segment is shiny and lightly chitinized and sepia-yellowish in colour.

Cocoon.—The caterpillar forms a roundish-ovalish cocoon well covered on all sides with larval frass, and it emits through its mouth a reddish juice which imparts a reddish tinge to the cocoon.

Pupa.—The pupa (Plate XXXVI, fig. 10) measures about 6 mm. in length and 2·25 mm. across the fourth abdominal segment. Its colour is yellowish-brown. The bases of the antennae are slightly raised or ridged and the ridges are joined by a central transverse ridge at the head. The first seven abdominal segments are distinct. The spiracles are small, oval, and darkish-brown in colour. They are situated laterally near the anterior margin of the abdominal segments. The abdomen has seven pairs of spiracles on the second to eighth segments, the pair on the last segment being closed.

The pupal period varies from nine to eighteen days depending on the season of the year (Table I).

TABLE I

Pupal period at the various times of the year

No.	Caterpillar collected on	Pupated	Emerged	Period in Days
1	13th December 1927.	19th December 1927.	3rd January 1928.	15
2	8th February 1929.	16th February 1929.	6th March 1929.	18
3	8th February 1929.	27th February 1929.	13th March 1929.	14
4	22nd March 1928.	26th March 1928.	6th April 1928.	11
5	11th July 1932.	Formed cocoon in the night of 11th July 1932. Pupated on 13th July 1932.	22nd July 1932.	9
6	3rd August 1932.	7th August 1932.	18th August 1932.	11

The moth at first sight appears to be a Geometrid but the hyaline, roundish patches on the fore-wings clearly demarcate it from that family (Plate XXXVI, fig. 11).

III. *RHODONEURA MYRSUSALIS* WLK.

The caterpillars were found feeding on *Achras sapota* (Sapotaceae) leaves on 18th September 1909 at Pusa, pupated on 19th September 1909 and the moths emerged on 26th September 1909. A caterpillar was also found rolling and feeding on leaf of *Bassia latifolia* (Sapotaceae) at Pusa early in September, 1920, the moth emerged on 11th September 1920. In the Pusa collection there is another moth of this species which was collected at Nagpur on 15th September 1908.

IV. *RHODONEURA MYRSUSALIS* WLK. VAR. *IDALIALIS*

The larva was found feeding on *Achras sapota* leaves at Pusa on 18th September 1909. The moth emerged on 26th September 1909.

V. *STRIGLINA SCITARIA* WLK.

The caterpillars were found feeding on *Dhaincha* (*Sesbania aculeata*) leaves at Coimbatore.

VI. *Dysodia ignita* Wlk.

The caterpillars were found between spun-up leaves of *Osbeckia* sp. at Shillong by Mr. Fletcher on 1st October 1916. One moth emerged from this lot on 7th March 1917. The larvae were also found on *Crataeva* (leaf ?) in Coimbatore, South India, on 3rd and 7th November 1914.

VII. *Dysodia viridatrix* Wlk.

The caterpillar was found on *Capparis diversifolia* stem (boring ?) by Y. Ramchandra Rao at Coimbatore (20th November 1916).

In conclusion, I wish to express my best thanks to Dr. Hem Singh Pruthi, the Imperial Entomologist, for kindly going through the manuscript and making many valuable suggestions.

REFERENCES

Fletcher, T. B. (1931). *Proc. Ent. Soc. London* **5**, pt. 3, p. 68.

Hampson, G. F. (1896). *Fauna of British India, Moths*, Vol. IV, p. 483.

——————— (1914). *Ann. Mag. Nat. Hist.* (8) **14**, p. 110.

Misra, C. S. (1919). *Proc. Third Ent. Meeting, Pusa*, Vol. II, p. 592.

Swinhoe, C. (1895). *Ann. Mag. Nat. Hist.* (6) **15**, p. 17.

Walker F. (1865). "List of the specimens of lepidopterous insects in the collection of the British Museum", XXXIII, p. 1111.

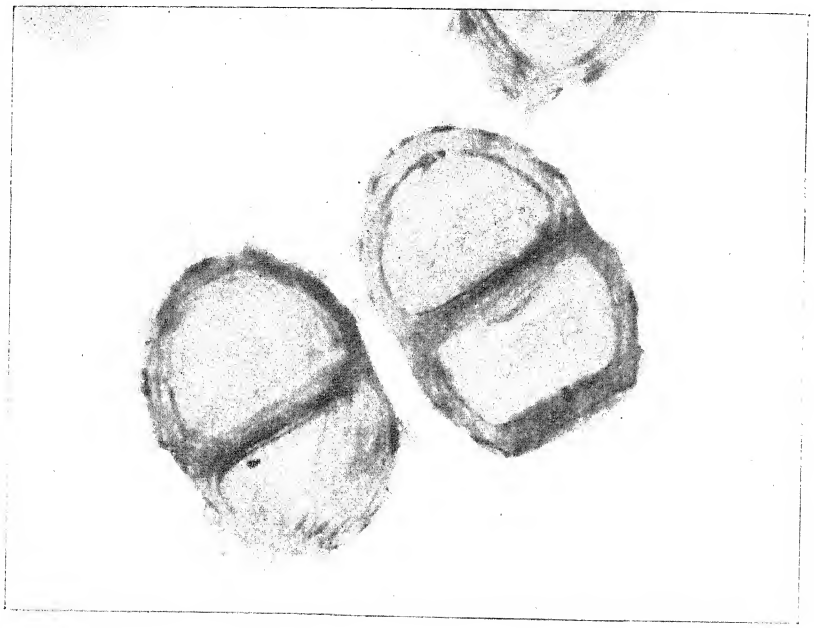


FIG. 1. Teleutospores of *P. prostii* (×900).

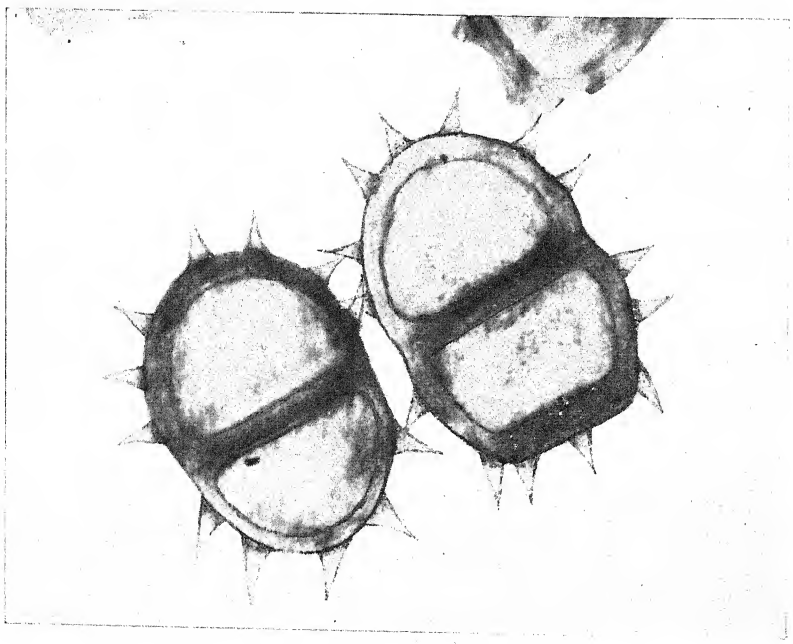


FIG. 2. Same as Fig. 1 retouched to show spines (×900).

MYCOLOGICAL NOTES

AN UNUSUAL RUST FUNGUS ON TULIP

BY

L. D. GALLOWAY, M.A.

AND

R. R. SEN

Imperial Institute of Agricultural Research, Pusa

(Received for Publication on 28th August 1935)

(With Plate XXXVII)

Among some specimens received at Pusa in May 1935 from the Agricultural Officer in Baluchistan, was a leaf of *Tulipa* sp. showing blackish sori at first covered and later bursting through longitudinal splits of the epidermis. The brown teleutospores, which were formed on both surfaces of the leaf, were borne on short pedicels and were covered with sub-hyaline spines 5-8μ in length. No spermogonia were found, but the teleutospores were unmistakably those of *Puccinia Prostii* Moug. A request for more material had to be set aside owing to the catastrophic earthquake at Quetta.

Puccinia Prostii with its very characteristic spiny teleutospores, does not appear to have been previously recorded for India. Saccardo [1888] states that it occurs on *Tulipa sylvestris* and *T. celsianae* in France and Italy. Massee [1913] gives a reference which suggests that it was recorded by him in Britain. Wilson [1914] gives a fuller description, with photographs, of the rust which he reports as doing considerable damage on *Tulipa sylvestris*. He mentions the production of spermogonia, which are not mentioned in previous descriptions.

The accompanying photomicrograph and re-touched photomicrograph (Plate XXXVII) show the characteristic structure of the spores. As regards their size, there is some variation in the literature quoted above. Saccardo gives the teleutospore size as 60-66 × 34-36μ, Massee as 54-66 × 34-40μ and Wilson as 56-62 × 17-19μ. This last measurement for width is an obvious error, as is shown by Wilson's own photographs.

Teleutospores of the Quetta specimen, measured in lacto-phenol, gave the following results :—

Mean of fifty measurements $53 \cdot 6 \times 37 \cdot 4 \mu$.

Range $44\text{-}66 \times 30\text{-}46 \mu$.

REFERENCES

Massee, G. (1913). 'Mildews, Rusts and Smuts'. London, 1913, p. 139.

Saccardo, P. A. (1888). *Sylloge Fungorum* **7**, 732.

Wilson, M. (1914). *Notes Roy. Bot. Garden, Edinburgh*, **38**, 219.

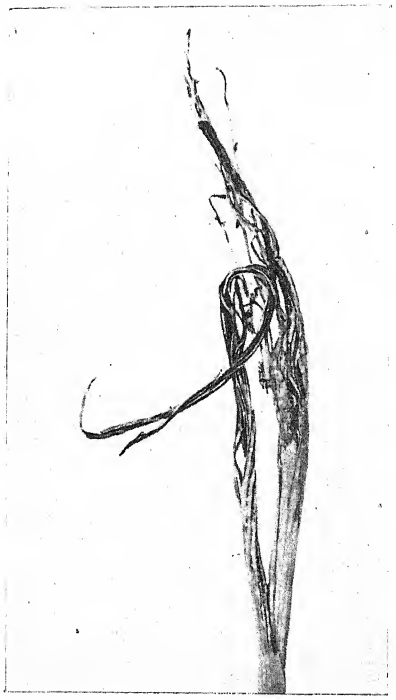


FIG. 1.

FIG. 2.

FIG. 3.

OAT LEAF INFECTION BY *USTILAGO AVENAE* (PERS) JENSEN

BY

B. B. MUNDKUR

Imperial Institute of Agricultural Research, Pusa

(Received for publication on 28th August 1935)

(With Plate XXXVIII)

The pathological effect usually produced by *U. avenae* on the oat plant is a complete destruction of the kernel and the enclosing glumes. The spores are disseminated as soon as the panicle emerges out of the boot and the central axis is thus completely exposed. This smut is rather rare in India, the more usual form being covered smut (*U. kolleri* Wille), but in the winter of 1934-1935 it appeared in Fulghum, Burt, Early Champion, Danish and Danish Island, oat varieties recommended by Reed [1927] as differentials in the determination of the physiologic races of covered smut and obtained from him.

On the variety Early Champion, Danish and Danish Island not only were the panicles affected but the upper leaves were also attacked. The sori developed on the blade of the flag leaf or on the leaf just below it; they occurred mostly on the ventral or upper side and were in the form of parallel striae. The sori were covered by the leaf tissue and the spores were released when this was ruptured. Many of the leaves became longitudinally shredded. Figures 1, 2, and 3 of Plate XXXVIII will make the above symptoms clear.

Exceptional cases of smut sori developing at unusual positions are well known. Jones [1896] has already recorded the infection of oat leaves by the loose smut and Lang [1913] has shown that he could produce the sori on the topmost leaves by checking their growth. Riehm [1914] observed a similar condition in the case of loose smut of wheat. Reed, Griffiths and Briggs [1925] found this condition to be rather prevalent in oat plants grown in the green-house, occasionally occurring in the field also. D'Almeida *et al*, quoted by Reed, Griffiths and Briggs [1925], seem to have considered the leaf-attacking form as a separate variety of smut which they have called *U. avenae* var. *foliicola*. At Pusa the condition was noted in the fields where growth of the leaves was not checked in any way.

REFERENCES

Jones, L. R. (1896). *Vt. Agric. Expt. Stn. 9th Ann. Rpt.*, 106-112.

Lang, W. (1913). *Jahresber. d. Ver. f. Ang. Bot.* **10**, 172-180.

Reed, G. M. (1927). *Mycologia* **19**, 21-28.

Reed, G. M., Griffiths, M.A. and Briggs, F. N. (1925). *U. S. Dept. Agric. Bull.* **1275**, 34-36.

Riehm. E. (1914). *Ber. d. Deutsch. Bot. Ges.* **32**, 570-573.

ABSTRACTS

Study of the evaporation of water from a soil surface with reference to the fluctuation of water-table. V. I. Vaidhianathan and Hans Raj Luthra.

(*Punjab Irrigation Research Institute Research Publication, Vol.* 5, *No. 3, November 1934*).

The ' Introduction ' and ' Summary ' are reproduced below :—

INTRODUCTION

During discussions of methods of cultivation to be undertaken in certain low rainfall areas in the Punjab, the question of the supply of water from a water-table to the surface arose. The conclusions arrived at by the earlier workers. regarding this question, were not definite. For example, Keen working at Rothamsted concluded that the upward capillary movement was ineffective even when the water-table is 80 cm. below the surface. In a later work, Keen mentions that water which has receded about 180 cm. is not drawn back by surface evaporation. McGee, from his investigations in the great planes of America, thought that water could rise at least 10 feet in a year and 30 to 35 feet in a favourable term of years. Hall suggested that soil moisture could rise to some 200 feet. Mitscherlisch made a calculation, which led to values of many hundreds of feet.

During certain previous investigations of the sub-soil water movement carried out in the Institute, it was observed that the water-table showed a fluctuation during each day in addition to a gradual fall in the hot season. It was thought that this fluctuation might be due to the evaporation of water from the surface during the hot portion of the day, the water from the water-table rising to the surface through the pores in the soil to replace that lost by evaporation. This would result in the lowering of the water-table. The fact that the water-table attained a maximum depth at about 13 hours supported such a suggestion. West working in Australia had observed that the sub-soil water-table showed a fluctuation depending on the atmospheric pressure. According to his conclusions when the atmospheric pressure increased, the level of the water-table went down and when the atmospheric pressure decreased, the level came up. The relation appeared to be so complete that no other factor seemed necessary to account for the fluctuation of the water-table. E. G. Bilham from his observations in a well at the Kew Observatory had also noticed that the sub-soil water-table fluctuated with the change of barometric pressure. Arrangements were made, therefore, to investigate the possible relation between the fluctuations of the water-table, evaporation, and barometric pressure.

The preliminary experiments were carried out in the Laboratory and the final ones in an area at the Lahore Cantonment. The latter area was not influenced by pumping and irrigation. The fluctuations of the water-table were therefore due only to natural causes. The month of June when the experiments were carried out was the most favourable period for the experiments, as it is the hottest and driest month in Lahore.

SUMMARY

1. The amount of water evaporating from the surface of the soil in an area near Lahore in the Punjab has been determined. It has been found that this is about $2 \cdot 7 \times 10^{-7}$ grms. per sq. cm. per second on the average for the month of June ; the temperature of the soil surface being about 66°C. during the hottest part of the day.

2. The dependence of the fluctuation of the water-table on surface evaporation and atmospheric pressure has been investigated. It has been found that while pressure has an effect on the fluctuation of the level of the water-table ; when the surface evaporation is high, the latter effect is the most predominating and the effect of pressure becomes negligible. The conclusions of the previous workers that pressure is the main factor affecting the level of the sub-soil water-table are not therefore found applicable to the conditions existing in the Punjab.

3. The moisture content of the soil from the water-table to the surface has been determined. It has been found that there is a continuity in the moisture content, though the gradient of the curve connecting the moisture content and the depth below the surface is not uniform.

4. The different results in the investigation have led to the conclusion that water is returning to the surface from a depth of 22 feet or 6·7 meters, which was the depth of the water-table in this investigation. The quantity that is being drawn up to the surface is too small for cultivation.

A soil moisture meter depending on the " Capillary pull " of the soil. With illustrations of its use in fallow land, grass orchard, and irrigated orchards. W. S. Rogers. (Horticultural Research Station, East Malling, Kent). The Journal of Agricultural Science (Cambridge), July 1935, Volume XXV, Part 3.

This paper describes a method for the continuous measurement of soil moisture which should be of very considerable use to research workers in India. The instrument was designed at the East Malling Station, has been in use there for over two years and has proved itself adaptable to a wide range of conditions. The author summarises his results in the following words :—

1. A soil moisture meter which gives direct and continuous measurement of the soil moisture content is described. The instrument consists of a special porous pot filled with water, connected by a tube to a mercury manometer. The pot is buried in the soil, whose capillary pull causes the mercury to rise. The height to which the

mercury rises depends on the amount of moisture in the soil, and also on the size of soil particles and the degree of compactness of the soil. (The last two factors remain constant for an instrument in one position).

2. To read actual moisture percentage each instrument has to be calibrated for the soil in which it is placed. Once this is done, all sampling and weighing is eliminated.

3. The range of the instrument in its present form runs from saturation to about 1·5 per cent moisture (calculated on dry weight) in sand, to about 8 per cent in light loam, and to about 21 per cent in heavy clay.

4. Within this range increases and decreases of soil moisture are recorded rapidly and consistently.

5. This type of moisture is prevented from giving absolutely precise readings of the soil moisture content owing to the fact that the moisture : pressure-deficiency curve tends to form a hysteresis loop, *i.e.*, does not follow exactly the same course for rising moisture as for falling moisture. The degree of accuracy appears to be sufficient for many purposes, however, for the variability is usually within 10 per cent.

6. Special devices are used to prevent freezing and enable the instrument to give a record over long periods without attention in the field.

7. Examples of the working of this moisture meter in the laboratory and in the field are given. Instruments placed at different depths in clean cultivated land and in grass orchards have shown the contrast between loss of water by evaporation, which hardly affects the soil moisture at 30 in. and loss by root absorption which draws on the deeper layers as well as the surface layers of the soil.

8. In an irrigated orchard the meters have satisfactorily shown the penetration of irrigation water and the drying out of the soil at various depths.

The original paper should be consulted for details. The appendix gives full particulars of the design and the method of construction and installation. Two forms of the complete instrument are now on the market.

NOTES

Standard Form of Certificate for Plant Imports into England, Scotland and Northern Ireland

It is notified for general information that His Majesty's Government in the United Kingdom have adopted the standard form of certificate given below for plant imports into England, Scotland and Northern Ireland.

CERTIFICATE

This is to certify that $\frac{\text{the living plants or plant products}}{\text{a representative sample of the living plants or plant products}}$

(*strike out the words not applicable*).

included in the consignment, of which particulars are given below were/was thoroughly examined on the (date).....by (name)..... a duly authorized official of the ..... and found to be healthy, no evidence of the presence of any injurious insect, pest, or disease(destructive to vegetation)

(destructive to agricultural or horticultural crops) having been [insert words in ( ) if required by existing legislation]

found in/on them and that the consignment (including the packing) covered by this certificate has/has not been treated in the following manner (*e.g.*, fumigated with.....or disinfected with.....) $\frac{\text{prior}}{\text{immediately subsequent}}$ to inspection.

Inspected in the field by a duly authorized inspector on.....

Not inspected

.....

(Signature).....

Official Status.....

Date ....

No. and description of packages.....

Distinguishing marks .....

Description of living plants or plant products or parts thereof.....

.....

Stated to be grown at.....

Exported by .....

Name and Address of consignee.....

Name of vessel or particulars of route.....

.....

Date of shipment.....

Port or place of entry.....

Additional Certificate(s) attached.....

LIST OF PUBLICATIONS OF THE IMPERIAL AGRICULTURAL BUREAUX (1934-35)

I. Obtainable from the Imperial Bureau of Soil Science, Rothamsted Experimental Station, Harpenden, Herts

[*All Publications are sent post free from the Imperial Bureau of Soil Science*]

1. Technical Communications

	Price
29. Soil, Vegetation and Climate. By G. V. Jacks. Pp. 43 . . .	2*s.*
30. The Determination of Exchangeable Bases in Soils. Pp. 35 . .	2*s.*
31. Soil Deficiencies and Plant Diseases. By G. V. Jacks and H. Scherbatoff. Pp. 48	2*s.*
32. Tea Soils. By Harold H. Mann. Pp. 66	2*s.*

2. Publications relating to Soils and Fertilizers

Monthly lists, with brief abstracts of all current papers and pamphlets indexed by the Bureau. The papers are classified by subjects, according to the Decimal Classification, and the lists are duplicated in a convenient form for cutting out the separate entries and filing them in an index, if required.

Price 10*s.* per annum, post free, separate copies each . . . . . 1*s.*

3. Monthly Letters

These consist of short accounts of outstanding pieces of recent research and of matters of general interest connected with the Bureau's activities. They are sent free, within the British Empire, to all recipients of Publications relating to Soils and Fertilizers. Outside the Empire, or when sold separately, a charge of 4*s.* per annum is made.

Separate copies, each . . . . . 6*d.*

4. Publications on Soil Science issued from the Empire Overseas

Bibliographical lists, 1933, 1934, each . . . . . 1*s.*

5. Recent Developments in Soil Analysis

A quarterly supplement to 'Publications Relating to Soils and Fertilizers,' containing full descriptions of recently published analytical methods, each . . . . . 6*d.*

6. Annual Reports

	Price
Nos. 5 and 6, each	6*d*.

7. Lists of Reprints available on loan

No. 1	1*s*. 6*d*.
Subsequent issues, each	6*d*.

8. Miscellaneous Publications

Bibliography of publications relating to Coffee Soils and Fertilizers (1934), pp. 55 . . . . . 2*s*.

9. Bibliography of Soil Science, Fertilizers and General Agronomy, 1931-34

A complete reference book to the recent literature of soil and allied sciences. The bibliography contains over 6,000 classified references, a comprehensive subject index, lists of some 4,000 authors and 800 journals and periodicals, and a concise account of the system of classification used.

Crown octavo, Pp. XXXI + 473. Bound in cloth . . . . . 25*s*.

Special Publication

The Katamorphism of Igneous Rocks under Humid Tropical Conditions. (By the late Professor Sir John Burchmore Harrison, C.M.G., Published on behalf of the British Association and Demerara Proprietors Ltd., this monograph gives a detailed account of the author's work, during thirty-seven years in British Guiana, on the processes of laterization).

Crown quarto ; 80 Pp., with a foreword by Sir E. J. Russell and a preface by Professor F. Hardy . . . . . 5*s*.

II. OBTAINABLE FROM THE IMPERIAL BUREAU OF PLANT GENETICS (FOR CROPS OTHER THAN HERBAGE), PLANT BREEDING INSTITUTE, SCHOOL OF AGRICULTURE, CAMBRIDGE

1. Journal

Plant Breeding Abstracts. Annual subscription . . . . . 15*s*.

2. Occasional Papers

Breeding Resistant Varieties, 1930-33 (Supplement) . . . . . 2*s*.

III. OBTAINABLE FROM THE IMPERIAL BUREAU OF PLANT GENETICS (HERBAGE PLANTS), WELSH PLANT BREEDING STATION, AGRICULTURAL BUILDINGS, ALEXANDRA ROAD, ABERYSTWYTH, WALES

1. *Herbage Abstracts*, which appears quarterly, deals with literature from all parts of the world on grassland and forage crop research and practice. An effort is made to reduce the interval between the appearance of a paper and of its abstract to a minimum.

2. *Herbage Reviews* represents the only Journal published in English which deals exclusively with grassland and forage crops. Contributions and correspondence are welcomed from all investigators on this subject, with the reservation that it is to be understood that no scientific paper representing the results of original (not yet published) research can be considered for publication.

Inclusive annual subscription . . . . . 15*s.*
Single numbers . . . . . 5*s.*

Occasional papers

Bulletins—

14. Grassland Research in Australia. Future Programme and contributions on pasture technique, Februray, 1934 . . . . . 3*s.*

15. Grassland and Forage Crops in Thuringia, Czechoslovakia and Hungary. By R. O. Whyte and Collaborators. August, 1934 . . . 3*s.* 6*d.*

16. The Theoretical Significance of Vernalization. By N. A. Maximov. December, 1934 . . . . . 2*s.* 6*d.*

IV. OBTAINABLE FROM THE IMPERIAL BUREAU OF FRUIT PRODUCTION, EAST MALLING RESEARCH STATION, EAST MALLING, KENT

Journal

Horticultural Abstracts.—A quarterly abstract publication of current horticultural literature. Vol. IV. Annual Subscription . . . . . 15*s.*
Single copy . . . . . 4*s.*

Technical Communication

5. The 'Degeneration' of the Strawberry, 1934. D. Akenhead, R. V. Harris, G. H. Berkeley, A. M. Massee . . . . . 2*s.*

Occasional Paper

Annotated Bibliography on Bitter-pit, 1934 . . . . . 1*s.* 6*d.*

Other Publications

Index to Vols. I-X of the *Journal of Pomology and Horticultural Science*, 1933, Compiled by Bureau, Published by the Editors of the *Journal of Pomology and Horticultural Science*. Available from the Bureau . . . . 5*s.*

V. OBTAINABLE FROM THE IMPERIAL BUREAU OF AGRICULTURAL PARASITOLOGY, INSTITUTE OF AGRICULTURAL PARASITOLOGY, WINCHES FARM DRIVE, HATFIELD ROAD, ST. ALBANS, HERTS.

Bibliography of Helminthology : for the year 1932 (including subject index to titles), 97 Pp. Library Edition, bound in cloth . . . . . 10*s.* 6*d.*
Stiff paper cover only . . . . . 8*s.*
Bibliography of Helminthology : for the year 1933 (including subject index to titles). Library Edition, bound in cloth . . . . . 10*s.* 6*d.*
Stiff paper cover only . . . . . 8*s.*

Helminthological Abstracts

Issued in six parts annually by the *Journal of Helminthology*; incorporates abstracts, prepared by the Bureau, of original papers on applied helminthology.

Subscription price for Vol. III (1934), post free . . . . . 30*s.* net

The Bearing of the Physiology of Parasitic Nematodes on their Treatment and Control (post free) . . . . . 3*s.*

VI. Obtainable from the Imperial Bureau of Animal Genetics, Institute of Animal Genetics, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh

Journal

Animal Breeding Abstracts (quarterly), commencing April 1933. Annual subscription . . . . . 15*s.*

Occasional Papers

Bibliography on the works of J. C. Ewart (free to subscribers of *Animal Breeding Abstracts, Vol. I.*), 1934 . . . . . 6*d.*

Animal Breeding in the British Empire. A Survey of Research and Experiment, 1934 . . . . . 2*s.*

VII. Obtainable from the Imperial Bureau of Animal Health, Veterinary Research Laboratory, New Haw, Weybridge, Surrey

Abstracting Journal

The Veterinary Bulletin: Annual subscription . . . . . 40*s.*

Indexing Publication

Index Veterinarius: Four issues a year. Annual subscription (postage paid) . . . . . 80*s.*

VIII. Obtainable from the Imperial Bureau of Animal Nutrition, Rowett Research Institute, Bucksburn, Aberdeen

Journal

Nutrition Abstracts and Reviews:—Issued under the direction of the Imperial Agricultural Bureaux Council, the Medical Research Council and the Reid Library. Subscription per Volume of 4 numbers . . . . . 21*s.*

Per single number . . . . . 6*s.* 6*d.*

Occasional Papers

Technical Communication

5. Recent Research in Poultry Nutrition, by A. R. G. Emslie . . 1*s.*

NOTICE

Copies of the under-mentioned publications are available for free distribution, provided the cost of packing and postage or railway freight is met by the indentor. Application should be made to the Secretary, Imperial Council of Agricultural Research (Publication Section), Imperial Record Department Building, New Delhi :—

1. Manual of more deadly forms of the cattle diseases in India (1903). 3rd Edition.
2. Notes on the cattle of Mysore, by Col. Pease (1911).
3. The indigenous breeds of cattle in Rajputana, by Col. Pease (1911).
4. The fodder grasses of N. India, by Duthie.
5. Some experiments in the treatment of surra in camel (1913).
6. Treatment of surra in horses by means of arsenic and its derivation.
7. Cure of surra in horses by the administration of arsenic.
8. Immunization against Charbon Symptomatique by means of a single vaccine (1909).
9. Report on the experiment carried out to test the susceptibility of cattle for several districts and on improved method of rinderpest serum preparation (1913).
10. Flax in India, by Robert S. Finlow (1918).
11. Catalogue of Ferns in the Herbarium of the Government of India at Saharanpur (1890).
12. Agriculture in India, by Mackenna.
13. Report of Mr. C. S. Marten on the Foreign Market for Indian Timber (1927).
14. Report on the extension of cinchona cultivation in India by Lt.-Col. Gage.
15. Catalogue of the "Plants of Kumaun," by Sir Richard Strachey and Duthie.
16. Report of the Proceedings of the Entomological Meeting, 2nd and 5th.
17. Review of Agricultural Operations in India, 1919-20 to 1921-22 and 1923-24 to 1928-29.

18. Report on the Progress of Agriculture in India, 1907-09, 1909-10, 1911-12 to 1918-19.

19. Memoirs of the Imperial Department of Agriculture in India.

(*a*) Botanical Series (*b*) Bacteriological Series (*c*) Chemical Series (*d*) Entomological Series (*e*) Veterinary Series.	Most of the back numbers are available and can be supplied as far as possible.
20. Agricultural Journal of India 21. Journal of the Central Bureau for Animal Husbandry and Dairying in India.	Ditto.

22. Notes on the collections and preservation of entomological specimens : Study of life history of insect, by Stebbing (1901).

GIPD—S2—206 ICofAR—6-1-36—800.